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(54) Title: TARGETING AND DELIVERY OF GENES AND ANTIVIRAL AGENTS INTO CELLS BY THE ADENOVIRUS PENTON

(57) Abstract

The present invention relates to gene therapy. In particular, therapeutic agents and methods useful in targeting and delivering non-native nucleotide sequences to specific cells are disclosed, wherein adenovirus penton, penton complex, or penton and fiber are used to facilitate targeting and delivery.

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TARGETING AND DELIVERY OF GENES
AND ANTIVIRAL AGENTS INTO CELLS
BY THE ADENOVIRUS PENTON

5   TECHNICAL FIELD

The present invention relates to gene therapy.
In particular, therapeutic agents and methods useful
in targeting and delivering genes to particular cells
are disclosed, wherein adenovirus penton (a complex of
10   penton base and fiber) or penton base is used to
facilitate targeting and delivery.

BACKGROUND

The primary impediment to the transfer of
non-native or foreign DNA into mammalian cells is that
15   the genetic material must be transported across
multiple cellular barriers before it enters the host
cell nucleus and initiates gene expression.
Previously established methods have utilized
artificial means to introduce DNA into the cell
20   although these methods are associated with significant
cell toxicity (Graham, et al., Virology 52: 456-467
(1973); Felgner, et al, PNAS USA 84: 7413-7417
(1987)).

This problem has been partially overcome by using
25   receptor-mediated endocytosis to deliver DNA into the
cell. In this procedure, a molecular conjugate
consisting of a ligand for a cell surface receptor
(i.e. transferrin) is covalently linked to a DNA
binding moiety (i.e., poly L-lysine) (Wu, et al., J.
30   Biol. Chem. 262: 4429-4432 (1987); Wagner, et al.,
PNAS USA 87: 3410-3414 (1990); and Wagner, et al.,
PNAS USA 88: 4255-4259 (1991)). The conjugate is thus
able to bind to the cell receptor and is subsequently
internalized by endocytosis. In many cases, however,
35   this approach is inefficient due to the targeting of
the DNA conjugate to lysosomes (Zenke, et al., PNAS

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USA 87: 3655-3659 (1990); Coqten, et al., PNAS USA 87:
4033-4037 (1990)). Although lysosomatropic agents can
sometimes be successfully used to inhibit this
process, a high degree of variability is often
5   observed in different cell types. (See, e.g., Curiel,
et al., PNAS USA 88: 8850-8854 (1991).)

        More recently, enhanced transfer of DNA
conjugates into cells has been achieved with
adenovirus, a human DNA virus which readily infects
10   epithelial cells (Horwitz, "Adenoviridae and their
replication", in Virology, Fields and Knipe, eds.,
Raven Press, NY (1990) pp. 1679-1740). Since
adenovirus efficiently disrupts the membranes of
endocytic vesicles, co-internalization of the virus
15   with the DNA conjugate allows rapid transfer of the
conjugate into the cell cytoplasm before it can be
subjected to lysosomal degradation. The fact that
adenovirus exhibits selective tropism for epithelial
cells has also been exploited to reconstitute these
20   cells in vivo with the human cystic fibrosis
transmembrane conductance regulator (CFTR) (Rosenfeld,
et al., Cell 68: 143-155 (1992)) and the alpha
1-antitrypsin genes (Rosenfeld, et al., Science 252:
431-434 (1991)). Adenovirus type 2 has also been
25   observed to facilitate the transfer of growth factors
or toxins from endosomes into the cytoplasm of
epithelial cells. (See, e.g., Fitzgerald, et al.,
Cell 32: 607-617 (1983); and Seth, et al., Mol. and
Cell. Biol. 4: 1528-1533 (1984).)
30   Other advantages of adenovirus-mediated gene
therapy include the efficient targeting of genes to
epithelial cells, and the efficient delivery of genes
to the cytoplasm, thus reducing lysosomal degradation
of DNA. Further, the use of replication-defective
35   strains of adenovirus reduces the cytopathic effect of

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replicating virus. Use of adenovirus may also
facilitate the transfer of DNA into host cells. In
vitro, adenovirus augments the delivery of
transferrin-poly L-lysine/DNA conjugates into host
5   cells. (See, e.g. Curiel, et al., _PNAS USA 88_:
8850-8854 (1991).) In vivo, alpha 1-antitrypsin and
cystic fibrosis transmembrane conductance regulator
protein genes have been delivered into epithelial
cells using adenovirus type 5 (Rosenfeld, et al.,
10   _Science 252_: 431-434 (1991); Rosenfeld, et al., _Cell_
_68_: 143-155 (1992)).

                    BRIEF SUMMARY OF THE INVENTION

        It has now been discovered that recombinant
adenovirus penton or penton base, independent of the
15   rest of the adenovirus genome, facilitates the
transfer of exogenous or non-native genes into
recipient cells. There are numerous advantages to the
use of adenovirus penton or penton base for gene
therapy over intact adenovirus or
20   replication-deficient adenovirus, particularly in
humans. For example, the adenovirus penton complex
likely possesses all of the functional properties
required for gene therapy including binding to
epithelial cell receptors, stimulation of endocytosis,
25   and penetration of endocytic vesicles. In addition,
large amounts of recombinant penton base and fiber may
be produced in insect cells via the use of an
expression system such as the baculovirus expression
system. The proteins so produced are capable of
30   assembling into the penton complex.

        Chimeric forms of recombinant penton complex may
also be constructed with the capacity to target
foreign genes to non-epithelial cells. These chimeric
forms are also capable of evading recognition and
35   attack by the host's immune system. Furthermore, the

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use of penton complex and fiber is a much safer
alternative for gene therapy applications than the
introduction of adenovirus genes into mammalian cells
as some forms of adenovirus contain genes that can

5   transform cells. Removal of those deleterious genes
from an adenovirus vector may not in itself be enough
to prevent transformation as the vector may combine
with a natural virus to produce a more pathogenic
strain. The use of recombinant or conventionally

10   purified penton base or penton complex of adenovirus
overcomes these secondary *in vivo* events.

Therefore, in one aspect, the present invention
contemplates a therapeutic agent capable of
specifically targeting epithelial cells comprising an

15   adenovirus penton base operatively linked to a
nucleotide sequence encoding a mammalian polypeptide
and an active promoter. In one embodiment, the
nucleotide sequence is conjugated directly to a region
of the penton which does not alter its functional

20   properties.

In various alternative embodiments, the
nucleotide sequence may be "sense" or "antisense". In
another embodiment, the penton base or penton complex
is incorporated into liposomes which contain the

25   foreign therapeutic nucleotide sequence or antisense
oligonucleotides. The invention also contemplates the
penton-mediated delivery of plasmid-based vectors
containing the gene of interest under the control of
strong constitutive or inducible promoters and/or

30   enhancer elements to obtain higher levels of gene
expression than that obtained by incorporating the
target gene into the intact adenovirus genome.

Thus, in one embodiment, the present invention
contemplates a composition designed to specifically

35   target epithelial cells and deliver a therapeutic

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nucleotide sequence to the cells, comprising an
adenovirus-derived protein and the nucleotide
sequence. In one variation, the protein is selected
from the group consisting of penton base and penton
5   complex. In the various embodiments contemplated
herein, the protein may be conventionally-purified or
recombinant, and may further be derived from
adenovirus type 2. The present invention further
contemplates that a composition may further comprise a
10   pharmaceutically acceptable carrier or excipient.

        In alternative embodiments, the adenovirus-
derived protein may be a complete protein, an
apoprotein, a polypeptide, or an amino acid residue
sequence comprising three or more amino acid residues
15   linked together via peptide linkages. In all
embodiments, the adenovirus-derived protein is
biologically active, and is preferably non-toxic.

        In another embodiment of the invention, the
therapeutic nucleotide sequence is operatively linked
20   to the adenovirus-derived protein. In another
variation, the therapeutic nucleotide sequence and the
adenovirus-derived protein are contained within a
liposome. In yet another embodiment, the nucleotide
sequence encodes a polypeptide and further comprises
25   an active promoter for expressing the polypeptide. In
variations thereof, the promoter is selected from the
group consisting of constitutive and inducible
promoters. In one variation, the nucleotide sequence
is a "sense" sequence; in another, the nucleotide
30   sequence is an "antisense" sequence.

        The present invention further contemplates the
use of a composition comprising an adenovirus-derived
protein and a therapeutic nucleotide sequence in the
manufacture of a medicament for specifically targeting
35   epithelial cells and delivering an effective amount of

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the therapeutic nucleotide sequence to the cells.

In another variation, the invention contemplates the use of a compound comprising a therapeutic nucleotide sequence operatively linked to an adenovirus-derived protein in the manufacture of a medicament for specifically targeting epithelial cells and delivering an effective amount of the therapeutic nucleotide sequence to the cells. In one embodiment, the adenovirus-derived protein is selected from the group consisting of penton base and penton complex. In other variations, it will be appreciated that any combination of the preceding elements may also be efficacious for the uses as described herein.

The invention further contemplates methods of making a medicament useful in specifically targeting and delivering a therapeutic nucleotide sequence to mammalian cells, comprising operatively linking an adenovirus-derived amino acid residue sequence and the nucleotide sequence and incorporating the operatively linked sequences into a liposome. In one variation, the amino acid residue sequence comprises penton base or penton complex. Other variations include combinations of elements as described hereinabove.

The present invention further contemplates an article of manufacture comprising packaging material and a composition effective for targeting epithelial cells and delivering a therapeutic nucleotide sequence to the cells, wherein the composition comprises an adenovirus-derived protein and a therapeutic nucleotide sequence, and wherein the packaging material comprises a label which indicates that the composition can be used for targeting epithelial cells and delivering a therapeutic nucleotide sequence to the cells.

In another embodiment, the invention contemplates

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a composition useful in specifically targeting non-epithelial cells and delivering a therapeutic nucleotide sequence to the cells, comprising an adenovirus-derived protein-ligand conjugate and the nucleotide sequence.

In yet another embodiment, the invention contemplates a composition designed to specifically target epithelial cells and deliver a therapeutic nucleotide sequence to the cells, comprising a protein or polypeptide including an RGD amino acid residue sequence, and the nucleotide sequence. In one variation, the composition is designed to specifically target endothelial cells. In yet another variation, the composition is designed to target malignant or tumor cells.

In various embodiments, the adenovirus-derived protein is selected from the group consisting of penton base and penton complex. In another embodiment, the composition is immunotherapeutic. In one variation, the composition is designed to target cells expressing integrins, including, in various embodiments, receptors for vitronectin, fibronectin, collagen, laminin, thrombospondin, fibrinogen, and von Willebrandt's factor. In one variation, the composition is designed to specifically target cells expressing a vitronectin receptor. In other variations, the composition is designed to specifically target cells expressing an $\alpha_v\beta_3$ or an $\alpha_v\beta_5$ receptor.

In various embodiments of the invention, the therapeutic nucleotide sequence is operatively linked to the adenovirus-derived protein. In another variation, the therapeutic nucleotide sequence and the adenovirus-derived protein are contained within a

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liposome.

In various embodiments, the nucleotide sequence
encodes a polypeptide and further comprises an active
promoter for expressing the polypeptide. In one
5   variation, the promoter is selected from the group
consisting of constitutive and inducible promoters.

In alternative embodiments of the invention, the
ligand may comprise an antibody, or may comprise an
attachment sequence for a receptor.

10   The present invention further contemplates a
method of specifically targeting and delivering a
therapeutic nucleotide sequence into mammalian cells,
comprising administration of a composition comprising
an adenovirus-derived protein and the nucleotide
15   sequence. In some variations, the adenovirus-derived
protein is selected from the group consisting of
penton base and penton complex. In various
embodiments, the adenovirus-derived protein is
recombinant; in others, the adenovirus-derived protein
20   is conventionally-purified. The invention further
contemplates that the adenovirus-derived protein is
derived from adenovirus type 2.

In alternative variations of the invention
contemplated herein, the therapeutic nucleotide
25   sequence is operatively linked to the adenovirus-
derived protein. In other embodiments, the
therapeutic nucleotide sequence and the adenovirus-
derived protein are contained within a liposome.

The invention further contemplates that compounds
30   and compositions of the present invention may be
administered in various forms and by various means,
including administration as an aerosol spray,
intraperitoneally, via surgical implantation or
injection, or via perfusion.

35   In various embodiments, a composition according

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to the present invention is administered in a dosage
range of 1 μg/ml to 1 mg/ml of active ingredient.

It will be appreciated that any combination of
the preceding elements may also be efficacious as

5   described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a schematic representation
of an adenovirus. The mobilities and relative amounts
of each protein after electrophoresis of the

10   dissociated virus on a sodium dodecyl
sulfate-containing polyacrylamide gel are shown on the
right. The position of each polypeptide in the virion
is designated; however, the configuration of the DNA
does not imply the actual structure within the core.

15   The Roman numerals refer to a polypeptide designation
described by Maizel, et al. (Virology 36: 126-136
(1968)). The hexon (II), penton base (III), fiber
(IV), and hexon-associated proteins (IIIa, VI, VIII,
and IX) are subunits of the capsid. The core contains

20   proteins V, VII, and u, as well as the 55 kD terminal
protein covalently linked at each of the 5' ends of
the linear DNA. The two molecules of TP per virion
are too few to be demonstrated by the Coomassie stain
of the viral polypeptides, and u probably is one of

25   the polypeptides found in the region of the gel
containing proteins X to XII. (See Persson, et al.,
Curr. Top. Microbiol. Immunol. 97: 157-203 (1982).)

Figure 2 (A and B) illustrates a diagram of the
adenovirus particle. Fig. 2A is a diagram

30   illustrating the adenovirus particle, showing the
location of major protein sub-assemblies. The nine
hexons drawn as a group and the "peripentonal" hexons
are trimers of the same polypeptide. They are
distinguished only by their location in the structure.

35   (See Burnett, in McPherson, et al. (eds.), Biological

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<u>Macromolecules and Assemblies</u>, Vol. I, "The Viruses",
Wiley, NY, pp. 337-385 (1984).) Fig. 2B illustrates a
cross-section, and shows the probable location of the
principal polypeptide components and the viral DNA.
The assignment for proteins other than II (hexon), III
(penton base), and IV (penton fiber) is based largely
on studies of stepwise dissociation. (See Ginsberg,
in Fraenkel-Conrat, et al. (eds.), <u>Comprehensive</u>
<u>Virology</u>, Vol. 13, Plenum, NY, pp. 409-457 (1979).)

      Figure 3 illustrates DNA delivery into cells in
culture via the recombinant penton base of adenovirus.
(Refer to Example 3 for details of the gene transfer
experiment.) Sample numbers are listed on the
horizontal axis, whereas luciferase activity (in light
units) is plotted on the vertical axis. The
abbreviation "PL" refers to poly L-lysine and pRSVLuc
is a reporter plasmid containing a luciferase gene.
The samples identified by numbers 1-5 contained the
following: (1) transferrin/PL, but no pRSVLuc; (2)
pRSVLuc + transferrin/PL; (3) pRSVLuc + transferrin/PL
+ penton base + fiber; (4) pRSVLuc + transferrin/PL +
penton base; (5) pRSVLuc + transferrin/PL + fiber.

                    DETAILED DESCRIPTION
A.   Definitions
      <u>Amino Acid Residue</u>: An amino acid formed upon
chemical digestion (hydrolysis) of a polypeptide at
its peptide linkages. The amino acid residues
described herein are preferably in the "L" isomeric
form. However, residues in the "D" isomeric form can
be substituted for any L-amino acid residue, as long
as the desired functional property is retained by the
polypeptide. NH$_2$ refers to the free amino group
present at the amino terminus of a polypeptide. COOH
refers to the free carboxy group present at the

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carboxy terminus of a polypeptide. In keeping with
standard polypeptide nomenclature (described in J.
Biol. Chem., 243:3552-59 (1969) and adopted at 37
C.F.R. §1.822(b)(2)), abbreviations for amino acid
5   residues are shown in the following Table of
Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		AMINO ACID
1-Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	cysteine
X	Xaa	Unknown or other

35   It should be noted that all amino acid residue

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sequences represented herein by formulae have a left
to right orientation in the conventional direction of
amino-terminus to carboxy-terminus. In addition, the
phrase "amino acid residue" is broadly defined to
5   include the amino acids listed in the Table of
Correspondence and modified and unusual amino acids,
such as those listed in 37 C.F.R. §1.822(b)(4), and
incorporated herein by reference. Furthermore, it
should be noted that a dash at the beginning or end of
10   an amino acid residue sequence indicates a peptide
bond to a further sequence of one or more amino acid
residues or to an amino-terminal group such as NH_2 or
to a carboxy-terminal group such as COOH.

DNA Homolog: A nucleic acid having a preselected
15   conserved nucleotide sequence and a sequence encoding
a preferred polypeptide according to the present
invention.

Downstream: Further along a DNA sequence in the
direction of sequence transcription or read out, that
20   is traveling in a 3'- to 5'-direction along the
non-coding strand of the DNA or 5'- to 3'-direction
along the RNA transcript.

Expression: The process undergone by a
structural gene to produce a polypeptide. It is a
25   combination of transcription and translation.

Gene: A nucleic acid whose nucleotide sequence
encodes an RNA or polypeptide. A gene can be either
RNA or DNA. Genes may include regions preceding and
following the coding region (leader and trailer) as
30   well as intervening sequences (introns) between
individual coding segments (exons).

Hybridization: The pairing of substantially
complementary nucleotide sequences (strands of nucleic
acid) to form a duplex or heteroduplex by the

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establishment of hydrogen bonds between complementary
base pairs. It is a specific, i.e. non-random,
interaction between two complementary polynucleotides
that can be competitively inhibited.

5   Ligand: A molecule that contains a structural
portion that is bound by specific interaction with a
particular receptor protein.

        Nucleotide: A monomeric unit of DNA or RNA
consisting of a sugar moiety (pentose), a phosphate
10   group, and a nitrogenous heterocyclic base. The base
is linked to the sugar moiety via the glycosidic
carbon (1' carbon of the pentose) and that combination
of base and sugar is a nucleoside. When the
nucleoside contains a phosphate group bonded to the 3'
15   or 5' position of the pentose, it is referred to as a
nucleotide. A sequence of operatively linked
nucleotides is typically referred to herein as a "base
sequence" or "nucleotide sequence", and their
grammatical equivalents, and is represented herein by
20   a formula whose left to right orientation is in the
conventional direction of 5'-terminus to 3'-terminus.

        Nucleotide Analog: A purine or pyrimidine
nucleotide that differs structurally from A, T, G, C,
or U, but is sufficiently similar to substitute for
25   the normal nucleotide in a nucleic acid molecule.

        Oligonucleotide or Polynucleotide: A polymer of
single or double stranded nucleotides. As used
herein, "oligonucleotide" and its grammatical
equivalents will include the full range of nucleic
30   acids. An oligonucleotide will typically refer to a
nucleic acid molecule comprised of a linear strand of
ribonucleotides. The exact size will depend on many
factors, which in turn depends on the ultimate
conditions of use, as is well known in the art.

35   Penton: The terms "penton" or "penton complex"

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are preferentially used herein to designate a complex
of penton base and fiber. The term "penton" may also
be used to indicate penton base, as well as penton
complex. The meaning of the term "penton" alone
should be clear from the context within which it is
used.

    Plasmid: An autonomous self-replicating
extrachromosomal circular DNA.

    Polypeptide and Peptide: These terms are used
interchangeably herein to designate a series of no
more than about 50 amino acid residues connected one
to the other by peptide bonds between the alpha-amino
and carboxy groups of adjacent residues.

    Protein: As used herein, this term designates a
linear series of greater than 50 amino acid residues
connected one to the other as in a polypeptide.

    Receptor: Receptor and receptor protein are
terms used herein to indicate a biologically active
proteinaceous molecule that specifically binds to (or
with) other molecules.

    Recombinant DNA (rDNA) molecule: A DNA molecule
produced by operatively linking a nucleic acid
sequence, such as a gene, to a DNA molecule sequence
of the present invention. Thus, a recombinant DNA
molecule may be a hybrid DNA molecule comprising at
least two nucleotide sequences not normally found
together in nature. rDNAs not having a common
biological origin, i.e., evolutionarily different, are
said to be "heterologous".

    Therapeutic Nucleotide Sequence: As described
and claimed herein, such a sequence includes DNA and
RNA sequences encoding an RNA or polypeptide. Such
sequences may be "native" or naturally-derived
sequences; they may also be recombinantly-derived
sequences. Therapeutic nucleotide sequences therefore

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include antisense sequences or nucleotide sequences
which may be transcribed into antisense sequences.
Therapeutic nucleotide sequences further comprise
sequences which function to produce a desired effect
in the cell or cell nucleus into which said
therapeutic sequences are delivered. For example, a
therapeutic nucleotide sequence may encode a
functional protein intended for delivery into a cell
which is unable to produce that functional protein.

Transfection: The acquisition of new genetic
markers by incorporation of added DNA in eucaryotic
cells.

Transformation: The acquisition of new genetic
markers by incorporation of added DNA in procaryotic
cells.

Upstream: In the direction opposite to the
direction of DNA transcription, and therefore going
from 5' to 3' on the non-coding strand, or 3' to 5' on
the mRNA.

Vector: A polynucleotide molecule capable of
autonomous replication in a cell and to which a DNA
segment, e.g., gene or polynucleotide, can be
operatively linked so as to bring about replication of
the attached segment.

Cloning Vector: Any plasmid or virus into which
a foreign DNA may be inserted to be cloned.

Expression Vector: Any plasmid or virus into
which a foreign DNA may be inserted or expressed.
Vectors capable of directing the expression of DNA
segments (genes) encoding one or more proteins are
referred to herein as "expression vectors". Also
included are vectors which allow cloning of cDNA
(complementary DNA) from mRNAs produced using reverse
transcriptase.

Leader or Signal Polypeptide: A short length of

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amino acid sequence at the amino end of a protein,
which carries or directs the protein through the inner
membrane and so ensures its eventual secretion into
the periplasmic space. The leader sequence peptide is
5   commonly removed before the protein becomes active.
        Reading Frame: A particular sequence of
contiguous nucleotide triplets (codons) employed in
translation. The reading frame depends on the
location of the translation initiation codon.
10   B.   Adenoviruses
        Since its discovery in 1953, adenovirus has
served as a model for molecular biology and cell
transformation. The pentagonal capsomer (the penton)
at the vertex of the adenovirus icosahedron consists
15   of a fiber projection, linked by noncovalent bonds to
the penton base, anchored in the capsid. (See, e.g.,
Novelli and Boulanger, Virology 185: 365-376 (1991);
Nermut, in The Adenoviruses, Ginsberg, ed., Plenum,
NY, pp. 5-34 (1984); and Pettersson, in The
20   Adenoviruses, Ginsberg, ed., Plenum, NY, pp. 205-207
(1984).)
        The adenovirus (Ad) particle is relatively
complex and may be resolved into various
substructures. The outer shell is strikingly
25   icosahedral in shape and, at first glance, appears to
have a triangulation number of 25. The structures at
the fivefold positions ("pentons") are different from
the rest ("hexons"), however, and the hexons are
chemically trimers rather than hexamers. Thus, the
30   structure really does not correspond to a simple
sub-triangulated icosahedral design. (See, e.g.,
Fields, et al., Virology, Vol. I, Raven Press, NY, pp.
54-56 (1990).)
        Adenoviruses are nonenveloped, regular
35   icosahedrons (20 triangular surfaces and 12 vertices)

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that are about 65-80 nm in diameter (about 1400
angstroms (Å)). A structure, called fiber, projects
from each of the vertices. The length of the fiber
varies with the adenovirus serotype. The protein coat

5   or capsid is composed of 252 subunits (capsomeres), of
which 240 are hexons and 12 are pentons. Each of the
pentons contains a penton base on the surface of the
capsid and a fiber projecting from the base, which is
surrounded by five hexons. The name "penton" is

10   derived from these geometric relationships. All the
other capsomeres are hexons, and they are so named
because each is surrounded by six neighboring
identical structures. A group of nine hexons (the
"ninemers") can be purified from each of the 20

15   triangular faces of the virion by gentle lysis of the
particles with 10% pyridine. The hexons and pentons
are each derived from different viral polypeptides.
Recently, combined electron-microscopic and
crystallographic approaches have produced detailed

20   descriptions of hexon-hexon and hexon-penton base
interactions that have helped clarify the relationship
of the virus subunits. In addition, the hexon
capsomere was shown to contain two ß barrel
configurations that were very similar to structures

25   contained on the surface of several picornaviruses
(poliovirus and rhinovirus). Disruption of the virion
either with 5M urea, 10% pyridine, acetone, or
multiple freeze-thaw cycles results in a core
structure composed of the double-strand DNA with four

30   additional viral proteins not appearing in the capsid.
Adenoviruses contain 13% DNA and 87% protein, have no
membranes or lipids, and are therefore stable in
solvents such as ether and ethanol. The virion
contains fiber polypeptides modified by covalent

35   addition of glucosamine and has a density of 1.34

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g/cm³ in CsCl.

    With regard to virion capsid polypeptides, most
of the detailed structural studies of adenovirus
polypeptides have been performed for Ad types 2 and 5.
5   The following discussion emphasizes Ad 2 data.
Variation in molecular weights of the analogous
proteins among the various serotypes is small but has
been useful in one of the adenovirus classification
schemes. In Fig. 1, the adenovirus polypeptides, as
10 well as their positions in the virion, are
illustrated. There is no polypeptide I because the
originally designated protein I was a mixture of
smaller molecules that formerly were difficult to
dissociate. Polypeptide II is the 120 kilodalton (kd)
15 hexon.

    The hexon capsomere is a trimer of three
identical 110K polypeptides, conventionally denoted
polypeptide II, held tightly together by noncovalent
interactions. The 240 hexon capsomeres in each virion
20 therefore contain 720 identical polypeptides. The
high-resolution structure shows that each subunit
contains two rather similar ß-barrel domains, in which
the course of the polypeptide chain is somewhat like
that in an RVC (RNA virus capsid) fold. That is, in
25 the hexon domains there are two sheets of four strands
each, connected sequentially as in the framework of an
RVC domain. However, the shape of the domain is
rather different; the strands run radially rather than
tangentially to the shell, and the major loops are
30 between strands D and E and strands F and G. Thus,
use of a similarly folded design may not imply any
evolutionary or functional relationship. The DE and
FG loops from both domains project outward,
interacting tightly with corresponding loops from the

other two subunits in the trimer. The conformation of
these loops therefore depends on trimer formation.

        The loops bear the principal type-specific
antigenic determinants. Assembly of the hexon from
newly synthesized protein *in vivo* appears to require a
factor, the "100K protein", which is also encoded by
the virus. This protein does not form part of the
final structure. Dissociation of adenovirus particles
by various methods yields groups of nine hexons, as
shown in Fig. 2A. They are derived from virions as
indicated, and they include all but the peripentonal
hexons. The groups of nine are held together by viral
protein IX (see Fig. 2B), which copurifies with these
structures. Its location has been determined by
scanning electron microscopy. (See, e.g., Fields, et
al., *Virology*, Vol. I, Raven Press, NY, pp. 54-56
(1990).)

        Polypeptides VI (24 kd), VIII (13 kd), and IX (12
kd) are associated with the hexon after various
isolation procedures. For example, after disruption
of the virion with 10% pyridine, polypeptides VI and
IX are isolated together with hexon ninemers.
Polypeptides VI and VIII are synthesized as larger
precursors and are cleaved during assembly. The
positions of these proteins within the virion are
shown in Fig. 1, and those polypeptides facing the
outer surface of the particle are indicated. The
location of polypeptides was obtained by surface
labeling of virion with iodinated lactoperoxidase or
by analysis of various degradation products of gently
disrupted particles. The penton base (polypeptide
III, 85 kd) is noncovalently attached to fiber
(polypeptide IV, 62 kd).

        The adenovirus penton is a noncovalently
associated complex of two proteins, the fiber and

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penton base. In general, the penton complex comprises
three fiber monomers associated with one penton base.
(See, e.g., van Oostrum and Burnett, _J. Virol. 56_:
439-448 (1985).) The complex of penton base plus
5   fiber polypeptides is called penton capsomere. There
are five molecules of polypeptide III per penton base,
as well as three molecules of polypeptide IV in the
fiber at each vertex. The outer tip of the penton
fiber is thought to be the site of attachment to
10   cellular receptors. The orientation of the fiber
polypeptide has been determined. The conserved amino
terminus is attached to the penton base, whereas the
knob containing the carboxy end projects farthest from
the capsid. There is a conserved structural motif in
15   the shaft of the fiber which contains periodic repeats
of prolines and hydrophobic residues. The amino acid
sequence of the shaft, however, is not well conserved,
which correlates with the antigenic variation of fiber
characteristic of the different virus types.
20   Polypeptide IIIa (66 kd) is associated with the
peripentonal hexons after pyridine dissociation of
virions. (See, e.g., Fields, et al., _Virology_, Vol.
II, Raven Press, NY, pp. 1681-1682 (1990).)
        The fiber is an elongated protein (180,000 kDa)
25   which exists as a trimer of three identical
polypeptides of 582 amino acids in length (62 kDa).
The N-terminus of the fiber mediates binding to the
penton base while the C-terminus is involved in
initial binding of the virus to cellular receptors.
30   Cellular receptors for the fiber have not yet been
identified. Adenovirus attachment to a putative host
cell receptor is mediated by the fiber protein as
demonstrated by the ability of soluble fiber to
completely block virus attachment and infection. The
35   elongated portion of the fiber (shaft) is comprised of

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22 repeats of 15 amino acids each (adenovirus type 2) which form amphipathic B sheet structure.

The adenovirus penton base is a pentameter comprised of 5 identical subunits of 571 amino acids which are noncovalently associated to form a ring-shaped complex of approximately 200 X 90 Å. There is a 98% sequence identity in the penton base within the same subgroup of adenoviruses while there is only 69% identity in the fiber. The penton base contains an arginine-glycine-aspartic acid-containing (RGD-containing) peptide sequence (i.e., HAIRGDTFA (SEQ ID NO 1), residues 340-342 within residues 337-345) which are now shown to be involved in the attachment of the virus to integrin receptors. Such binding is required for internalization of adenovirus into the host cell. Synthetic peptides containing the RGD sequence inhibit virus infection as well as virus entry into the host cell. Thus, adenovirus, which is a nonenveloped DNA virus, utilizes separate proteins for attachment and entry in a manner similar to enveloped human viruses.

Adenoviruses attach to host-cell receptors via the penton fiber glycoprotein and enter cells through the process of receptor-mediated endocytosis mediated by the penton base. The penton fiber projects from the vertices of the virion capsid. It has a small distal knob that contains the cell attachment site. It has been estimated that there are 10^4 virion binding sites per cell. There are a significantly larger number of cellular binding sites for the isolated penton fiber protein, indicating that the intact virion occupies several receptor sites. Antibodies directed against the penton fiber neutralize viral infectivity. Affinity columns made

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with the adenovirus penton capsomer have been used to
isolate and partially purify the receptor for
adenovirus on KB cells. Three proteins of molecular
mass 34, 42, and 78 kd have been isolated using this
5   technique. These proteins have been presumed to
comprise different forms of the same molecule or
polypeptides which together form a part of a receptor
complex.

The 35,000 base pair (bp) genome of adenovirus
10   type 2 has been sequenced and the predicted amino acid
sequences of the major coat proteins (hexon, fiber and
penton base) have been described. (See, e.g., Neumann
et al., _Gene_ 69:153-157 (1988); Herisse et al., _Nuc._
Acids Res. 9:4023-4041 (1981); Roberts et al., _J._
15   _Biol. Chem._ 259:13968-13975 (1984); Kinloch et al., _J._
Biol. Chem. 259:6431-6436 (1984); and Chroboczek et
al., _Virol._ 161:549-554 (1987).) The core of
adenovirions contains DNA (about 30 kb) and two basic
proteins (V and VII). The arginine-rich protein VII
20   is present in about 1070 copies/particle, and it can
neutralize about 50% of the DNA phosphates. Isolated
cores are compact particles, but without any very
striking substructures.

Adenovirus DNA is about 23.85×10^6 daltons for
25   adenovirus type 2 (Ad 2) and varies slightly in size,
depending on serotype. Ad DNA is approximately 11
micromolar (μm) in length and has the unusual feature
of a virus-encoded 55 kd terminal polypeptide (TP)
covalently linked to dCMP at each 5' end of the linear
30   genome. Extensive proteolysis does not remove all the
amino acids from the 5' terminal dCMP; however, the
protein-DNA bond involving a serine hydroxyl group is
alkaline-labile in sodium hydroxide or piperidine.
Because TP does not allow the covalently linked

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fragments of DNA to enter agarose gels during
electrophoresis without detergents, the end fragments
of DNA can easily be determined for new serotypes.

5   Via electron microscopy, approximately half the
Ad DNA molecules released from virions with 4M
guanidine can be seen as circles formed by noncovalent
interactions between the terminal proteins at each
end. Adenovirus DNA has inverted terminal
10   redundancies and the 100- to 140-base-pair length of
these repeats varies with the serotype. Therefore,
denatured single strands of adenovirus DNA can form
circles by base pairing at the ends of the DNA, and
these "panhandle" structures may be important in DNA
replication.

15   Extensive restriction endonuclease maps exist for
many Ad serotypes. The base compositions of some Ad
serotypes vary from 48% to 61% G+C content, and this
is one criterion for classifying the human
adenoviruses. Considerable DNA sequencing data are
20   currently available, especially for Ad serotypes 2, 5,
7, and 12 (Baum, et al., _J. Virol. 10_: 211-219 (1972);
Tooze, _DNA Tumor Viruses: Molecular Biology of Tumor_
Viruses, 2d ed., Cold Spring Harbor Laboratory, NY,
pp. 937-1036 (1981); van Ormondt, et al., _Curr. Top._
25   _Microbiol. Immunol. 110_: 73-142 (1984)).

    Although adenovirus-mediated gene therapy
represents an improved method of DNA transfer into
cells, a potential limitation of this approach is that
adenovirus replication results in disruption of the
30   host cell. In addition, adenovirus also possesses
oncogenic properties including the ability of one of
its proteins to bind to tumor suppressor gene
products. The use of replication defective strains of
adenovirus which render the virus unable to replicate
35   in host cells is in principle more suitable for _in_

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vivo therapy; however, the potential of co-infection
of epithelial cells with wild-type strains of virus
resulting in transactivation of the recombinant virus
may represent a significant safety concern for *in vivo*
5   applications. Furthermore, it is not yet known
whether recombinant adenoviruses are capable of
integrating their genome into host cell DNA allowing
for long-term stable expression of the foreign gene.

       Another undesirable aspect of using intact or
10   replication-deficient adenovirus as a gene transfer
means is that it is an oncogenic virus whose gene
products are known to interfere with the function of
host cell tumor suppressor proteins as well as immune
recognition molecules, such as the major
15   histocompatibility complex (MHC). In addition,
pre-existing circulating antibodies to adenovirus may
significantly reduce the efficiency of *in vivo* gene
delivery. Lastly, only a foreign gene of 6 kilobases
(kb) or less can be incorporated into the intact
20   adenovirus genome for gene transfer experiments while
DNA segments of greater than 15 kb can be transferred
using the methods of this invention.

       The present invention uses a coat protein subunit
of adenovirus known as the penton which duplicates
25   cell receptor binding and DNA delivery properties of
intact adenovirus virions and thus represents an
improved method for gene therapy as well as
antisense-based antiviral therapy. The penton is
composed of a fiber protein which mediates receptor
30   binding and the penton base (Boudin, et al., Virology
116: 589-604 (1982)) which mediates various entry and
also disruption of the endocytic vesicle following
exposure to a low pH environment thus allowing virus
entry into the cytoplasm (Seth, et al., J. Biol. Chem.
35   260: 9598-9602 (1985); Seth, et al., J. Biol. Chem.

259: 14350-14353 (1984)).

In contrast to the disadvantages of using intact
or replication-deficient adenovirus, the use of
adenovirus-derived penton base and fiber proteins
5 provides certain advantages for gene delivery. The
adenovirus penton complex likely possesses all of the
functional properties required for gene therapy
including binding to epithelial cell receptors and
penetration of endocytic vesicles. Second, while
10 penton base and fiber proteins can be purified
directly from adenovirus using conventional
purification techniques as described in Example 2,
large amounts of recombinant penton base and fiber can
be produced in insect cells using baculovirus as well
15 as from other various expression systems following
amplification of genes encoding the penton base and
fiber. While the adenovirus type 2-derived
recombinant penton base and fiber are preferred for
use in this invention, the fiber and penton base
20 proteins purified by conventional methods from
adenovirus as described in Example 2 are also
contemplated for use in this invention.

The recombinant proteins can be amplified from
the available human adenovirus serotypes from type 1
25 through 47 currently available from American Type
Culture Collection (ATCC), Rockville, MD. Both
recombinant and conventionally purified penton base
and fiber proteins are capable of assembling into the
penton complex. Use of adenovirus penton also
30 eliminates the safety concerns related to the
introduction of intact adenovirus genes into human
cells.

Recombinant or conventionally purified adenovirus
type 2 penton as well as the noncomplexed penton base
35 and fiber proteins used independently are used in this

invention to facilitate specific targeting and
delivery of foreign genes into epithelial cells. For
this purpose, DNA or antisense-based plasmids are
conjugated directly to a region of the penton such
5   that its functional properties are not altered.
Alternatively, the penton complex, penton base or
fiber is incorporated into liposomes which contain the
foreign gene or antisense oligonucleotides. The
invention also uses plasmid-based vectors containing
10   the gene of interest under the control of strong
constitutive or inducible promoters and/or enhancer
elements as described in Example 5 to obtain higher
levels of gene expression than that obtained by
incorporating the target gene into the adenovirus
15   genome.

An extension of this invention is that the
adenovirus penton will be used to target and deliver
genes into non-epithelial cells by incorporating the
attachment sequence for other receptors such as CD4 on
20   T cells onto the fiber protein by recombinant DNA
techniques, thus producing a chimeric molecule as
described in Example 5. This should result in the
ability to target and deliver genes into a wide range
of cell types with the advantage of evading
25   recognition by the host's immune system. The
penton-based gene delivery system thus provides for
increased flexibility in gene design to enable stable
integration into proliferating and nonproliferating
cell types. For example, this is contemplated by the
30   incorporation of a retrovirus genome into the
penton-based gene delivery system, the result of which
allows for the stable integration of the exogenous
gene into the recipient cell mediated by the
retrovirus while allowing for delivery into
35   nonproliferating cells mediated by the penton. Gene

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transfer mediated by retroviruses outside this cont xt
is limited to proliferating cell types. For a review
of gene transfer by using retroviral vectors, see
International Applications WO 92/07943 and WO
5   92/07959, the disclosures of which are hereby
incorporated by reference. Thus, increased
flexibility and enhanced stable gene transfer is
attained by coupling the two gene transfer systems.

One application of this invention is gene therapy
10   for common hereditary disorders involving respiratory
epithelium such as alpha 1-antitrypsin deficiency
resulting in emphysema and cystic fibrosis
transmembrane conductance regulator (CFTR) deficiency
resulting in cystic fibrosis. A second application is
15   delivery of antiviral agents such as antisense
compounds or ribozymes to epithelial cells as
described in Example 5. The adenovirus penton may
also be useful in the enhanced delivery of
immunotoxins for the treatment of various cancers.

20   C.   Expression and Purification of Recombinant Penton
Base and Fiber

1.   PCR Amplification of Penton Base and Fiber
Proteins

Using PCR, it is possible to synthesize
25   useful polypeptide-encoding nucleotide sequences which
may then be operatively linked to a vector and used to
transform an appropriate cell and expressed therein.

Particularly preferred methods for producing
large quantities of recombinant penton base and fiber
30   proteins of the present invention rely on the use of
preselected oligonucleotides as primers in a
polymerase chain reaction (PCR) to form PCR reaction
products as described herein. PCR is also used in the
preparation of the therapeutic compositions of this
35   invention as described below. For this embodiment,

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preparation of genes or nucleotide sequences for
therapeutic gene delivery is typically accomplished by
primer extension, preferably by primer extension in a
polymerase chain reaction (PCR) format.

5   If the DNA products described above are to be
produced by (PCR) amplification, two primers, i.e., a
PCR primer pair, must be used for each coding strand
of nucleic acid to be amplified. The first primer
becomes part of the nonsense (minus or complementary)
10   strand and hybridizes to a nucleotide sequence
conserved among the preferred gene's plus (or coding)
strands. To produce coding DNA homologs, first
primers are therefore chosen to hybridize to (i.e. be
complementary to) conserved regions within the gene(s)
15   of choice.

          Second primers become part of the coding (plus)
strand and hybridize to a nucleotide sequence
conserved among minus strands. To produce the coding
DNA homologs, second primers are therefore chosen to
20   hybridize with a conserved nucleotide sequence at the
5' end of the coding gene such as in that area coding
for the leader or first framework region. It should
be noted that in the amplification of the coding DNA
homologs the conserved 5' nucleotide sequence of the
25   second primer can be complementary to a sequence
exogenously added using terminal deoxynucleotidyl
transferase as described by Loh et al., _Science 243_:
217-220 (1989). One or both of the first and second
primers can contain a nucleotide sequence defining an
30   endonuclease recognition site (restriction site). The
site can be heterologous to the gene being amplified
and typically appears at or near the 5' end of the
primer.

          The first primer of a PCR primer pair is
35   sometimes referred to herein as the "sense primer"

because it hybridizes to the coding or sense strand of
a nucleic acid. In addition, the second primer of a
PCR primer pair is sometimes referred to herein as the
"antisense primer" because it hybridizes to a

5   non-coding or antisense strand of a nucleic acid,
i.e., a strand complementary to a coding strand. A
plurality of first primers and/or a plurality of
second primers can be used in each amplification,
e.g., one species of first primer can be paired with a

10 number of different second primers to form several
different primer pairs. Alternatively, an individual
pair of first and second primers can be used. Primers
are also referred to as being either 5' or 3' primers
indicating the ends or region of the DNA to which the

15 primers hybridize. In this case, the 5' and 3' primers
are respectively the antisense and sense primers.

     When present, the restriction site-defining
portion is typically located in a 5'-terminal
non-priming portion of the primer. The restriction

20 site defined by the first primer is typically chosen
to be one recognized by a restriction enzyme that does
not recognize the restriction site defined by the
second primer, the objective being to produce a DNA
molecule having cohesive termini that are

25 non-complementary to each other and thus allow
directional insertion into a vector.

     In PCR, each primer works in combination with a
second primer to amplify a target nucleic acid
sequence. The choice of PCR primer pairs for use in

30 PCR is governed by various considerations, as
discussed herein. That is, the primers have a
nucleotide sequence that is complementary to a
sequence conserved in the gene of choice. Useful
priming sequences are disclosed hereinafter.

35   The strategy used for cloning the selected genes

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will depend, as is well known in the art, on the type,
complexity, and purity of the nucleic acids making up
the various genes. Other factors include whether or
not the genes are to be amplified and/or mutagenized.

5   In general, the exemplary genes are comprised of
polynucleotide coding strands, such as mRNA and/or the
sense strand of genomic DNA. If the polynucleotide
sequence is in the form of double stranded genomic
DNA, it is usually first denatured, typically by

10   melting, into single strands. A gene sequence is
subjected to a PCR reaction by treating (contacting)
the sequence with a PCR primer pair, each member of
the pair having a preselected nucleotide sequence.
The PCR primer pair is capable of initiating primer

15   extension reactions by hybridizing to nucleotide
sequences, preferably at least about 10 nucleotides in
length and more preferably at least about 20
nucleotides in length, conserved within the gene
sequence.

20   The PCR reaction is performed by mixing the PCR
primer pair, preferably a predetermined amount
thereof, with the nucleic acids of the selected gene
or DNA nucleotide sequence, preferably a predetermined
amount thereof, in a PCR buffer to form a PCR reaction

25   admixture. The admixture is maintained under
polynucleotide synthesizing conditions for a time
period, which is typically predetermined, sufficient
for the formation of a PCR reaction product, thereby
producing a plurality of different

30   polypeptide-encoding DNA homologs.
       The PCR reaction is performed using any suitable
method. Generally it occurs in a buffered aqueous
solution, i.e., a PCR buffer, preferably at a pH of
7-9, most preferably about 8. Preferably, a molar

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excess (for genomic nucleic acid, usually about $10^6:1$
primer:template) of the primer is admixed to the
buffer containing the template strand. A large molar
excess is preferred to improve the efficiency of the
5   process.

The PCR buffer also preferably contains the
deoxyribonucleotide triphosphates dATP, dCTP, dGTP,
and dTTP and a polymerase, typically thermostable, all
in adequate amounts for primer extension
10   (polynucleotide synthesis) reaction. The resulting
solution (PCR admixture) is heated to about 90°C -
100°C for about 1 to 10 minutes, preferably from 1 to
4 minutes. After this heating period the solution is
allowed to cool to 54°C, which is preferable for
15   primer hybridization. The synthesis reaction may
occur at room temperature up to a temperature above
which the polymerase (inducing agent) no longer
functions efficiently. Thus, for example, if DNA
polymerase is used as inducing agent, the temperature
20   is generally no greater than about 40°C. An exemplary
PCR buffer comprises the following: 50 mM KCl; 10 mM
Tris-HCl at pH 8.3; 1.5 mM $MgCl_2$; 0.001% (wt/vol)
gelatin, 200 μM dATP; 200 μM dTTP; 200 μM dCTP; 200 μM
dGTP; and 2.5 units *Thermus aquaticus* DNA polymerase I
25   (U.S. Patent No. 4,889,818) per 100 microliters of
buffer. PCR for amplifying the recombinant penton
base and fiber proteins of this invention was
performed as described in Example 1.

The inducing agent may be any compound or system
30   which will function to accomplish the synthesis of
primer extension products, including enzymes.
Suitable enzymes for this purpose include, for
example, *E. coli* DNA polymerase I, Klenow fragment of
E. coli DNA polymerase I, T4 DNA polymerase, other

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available DNA polymerases, reverse transcriptase, and
other enzymes, including heat-stable enzymes, which
will facilitate combination of the nucleotides in the
proper manner to form the primer extension products
5   which are complementary to each nucleic acid strand.
Generally, the synthesis will be initiated at the 3'
end of each primer and proceed in the 5' direction
along the template strand, until synthesis terminates,
producing molecules of different lengths. There may
10   be inducing agents, however, which initiate synthesis
at the 5' end and proceed in the above direction,
using the same process as described above.

     The inducing agent also may be a compound or
system which will function to accomplish the synthesis
15   of RNA primer extension products, including enzymes.
In preferred embodiments, the inducing agent may be a
DNA-dependent RNA polymerase such as T7 RNA
polymerase, T3 RNA polymerase or SP6 RNA polymerase.
These polymerases produce a complementary RNA
20   polynucleotide. The high turn-over rate of the RNA
polymerase amplifies the starting polynucleotide and
has been described by Chamberlin et al., The Enzymes,
ed. P. Boyer, PP. 87-108, Academic Press, New York
(1982). Another advantage of T7 RNA polymerase is
25   that mutations can be introduced into the
polynucleotide synthesis by replacing a portion of
cDNA with one or more mutagenic oligodeoxynucleotides
(polynucleotides) and transcribing the
partially-mismatched template directly as has been
30   previously described by Joyce et al., Nuc. Acid Res.
17: 711-722 (1989). Amplification systems based on
transcription have been described by Gingeras et al.,
in PCR Protocols, A Guide to Methods and Applications,
pp 245-252, Academic Press, Inc., San Diego, CA
35   (1990).

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If the inducing agent is a DNA-dependent RNA
polymerase and therefore incorporates ribonucleotide
triphosphates, sufficient amounts of ATP, CTP, GTP and
UTP are admixed to the primer extension reaction
5   admixture and the resulting solution is treated as
described above.

The newly synthesized strand and its
complementary nucleic acid strand form a
double-stranded molecule which can be used in the
10   succeeding steps of the process.

After producing various polypeptide-encoding DNA
homologs for one or a plurality of different genes or
DNA segments, the DNA molecules are typically further
amplified. While the DNA molecules can be amplified
15   by classic techniques such as incorporation into an
autonomously replicating vector, it is preferred to
first amplify the molecules by subjecting them to a
polymerase chain reaction (PCR) prior to inserting
them into a vector. PCR is typically carried out by
20   thermocycling i.e., repeatedly increasing and
decreasing the temperature of a PCR reaction admixture
within a temperature range whose lower limit is about
10°C to about 40°C and whose upper limit is about 90°C
to about 100°C. The preferred amplification procedure
25   was performed as described in Example 1. The
increasing and decreasing can be continuous, but is
preferably phasic with time periods of relative
temperature stability at each of temperatures favoring
polynucleotide synthesis, denaturation and
30   hybridization.

PCR amplification methods are described in detail
in U.S. Patent Nos. 4,683,192, 4,683,202, 4,800,159,
and 4,965,188, and at least in several texts including
"PCR Technology: Principles and Applications for DNA
35   Amplification", H. Erlich, ed., Stockton Press, New

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York (1989); and "PCR Protocols: A Guide to Methods
and Applications", Innis et al., eds., Academic Press,
San Diego, California (1990). Various preferred
methods and primers used herein are described

5   hereinafter and are also described in Nilsson, et al.,
Cell 58: 707 (1989), Ennis, et al., PNAS USA 87:
2833-7 (1990), and Zemmour, et al., Immunogenetics 33:
310-20 (1991), for example. In particular for
amplifying the therapeutic nucleotide sequences for

10   use in this invention, it is preferred to design
primers from comparison of 5' and 3' untranslated
regions of known allelic forms, with selection of
conserved sequences. Restriction sites may also be
incorporated into the 5' and 3' primers to enable the

15   amplification products to be subcloned into sequencing
or expression vectors. It may also be helpful to
place a 4-base spacer sequence proximal to the
restriction site to improve the efficiency of cutting
amplification products with enzymes.

20   The following primers are preferred for
amplification of penton base and fiber cDNA from
adenovirus type 2 DNA, preferably in separate
reactions. The adenoviral template DNA is obtained as
described in Example 1. Resulting cDNAs may then be

25   cloned and sequenced as described herein. These
primers are appropriate for use in amplifying all
known and presently unknown types of adenovirus penton
base and fiber.

         The 5' and 3' primers used to amplify the penton

30   base were, respectively, 5'-TTTCTAGAAGTATGCAGCGCGCG-3'
(SEQ ID NO 2) and 5'-TTTCTAGATCAAAAAGTGCGGCT-3' (SEQ
ID NO 3). The 5' and 3' primers used to amplify the
fiber were, respectively,
5'-AAAGGATCCAGCTGATGAAACGCGCCA-3' (SEQ ID NO 4) and

35   5'-TTTGGTACCAGCTGTTATTCCTGGGCA-3' (SEQ ID NO 5). The

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restriction endonuclease cloning sites indicated by
the underlined nucleotides are described in Example 1.

In preferred embodiments only one pair of first
and second primers is used per amplification reaction.
The amplification reaction products obtained from a
plurality of different amplifications, each using a
plurality of different primer pairs, are then
combined. However, the present invention also
contemplates DNA homolog production via
co-amplification (using two pairs of primers), and
multiplex amplification (using up to about 8, 9 or 10
primer pairs).

2.   Expression Vector Systems for Expression of
     Penton Base and Fiber Proteins

Expression of recombinant penton base and fiber
proteins of this invention is accomplished through the
use of expression vectors into which the PCR amplified
penton base or fiber sequences described above have
been inserted. The expression vectors may be
constructed utilizing any of the well-known vector
construction techniques. Those techniques, however,
are modified to the extent that the translatable
nucleotide sequence to be inserted into the genome of
the host cell is flanked "upstream" of the sequence by
an appropriate promoter and/or enhancer sequences.

In a preferred embodiment, the vector also
contains a selectable marker. After expression, the
product of the translatable nucleotide sequence may
then be purified using antibodies against that
sequence. One example of a selectable marker is
neomycin resistance. A plasmid encoding neomycin
resistance, such as *phshsneo*, *phsneo*, or *pcopneo*, may
be included in each transfection such that a
population of cells that express the gene(s) of choice
may be ascertained by growing the transfectants in

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selection medium.

In a preferred embodiment, the translatable
nucleotide sequence may be incorporated into a plasmid
with an appropriate controllable transcriptional
5   promoter, translational control sequences, and a
polylinker to simplify insertion of the translatable
nucleotide sequence in the correct orientation, and
may be expressed in the host cells. Various host
cells include a eucaryotic insect cell, such as
10   *Spodoptera frugiperda*, or a procaryotic cell, such as
E. coli. Preferably, there are 5' control sequences
defining a promoter for initiating transcription and a
ribosome binding site operatively linked at the 5'
terminus of the upstream translatable DNA sequence.

15   To achieve high levels of gene expression in
transformed or transfected cells -- for example, *E.
coli* -- it is necessary to use not only strong
promoters to generate large quantities of mRNA, but
also ribosome binding sites to ensure that the mRNA is
20   efficiently translated. In *E. coli*, for example, the
ribosome binding site includes an initiation codon
(AUG) and a sequence 3-9 nucleotides long located 3-11
nucleotides upstream from the initiation codon (Shine
et al., *Nature*, 254:34 (1975)). The sequence,
25   AGGAGGU, which is called the Shine-Dalgarno (SD)
sequence, is complementary to the 3' end of *E. coli*
16S mRNA. Binding of the ribosome to mRNA and the
sequence at the 3' end of the mRNA can be affected by
several factors, including (1) the degree of
30   complementarity between the SD sequence and 3' end of
the 16S tRNA; and (2) the spacing and possibly the DNA
sequence lying between the SD sequence and the AUG.
(See, e.g., Roberts et al., *PNAS USA 76*: 760 (1979a);
Roberts et al., *PNAS USA 76*: 5596 (1979b); Guarente et
35   al., *Science 209*: 1428 (1980); and Guarente et al.,

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Cell 20: 543 (1980).)

     Optimization is generally achieved by measuring
the level of expression of genes in plasmids in which
this spacing is systematically altered. Comparison of
different mRNAs shows that there are statistically
preferred sequences from positions -20 to +13 (where
the A of the AUG is position 0; see, e.g., Gold et
al., Ann. Rev. Microbiol. 35: 365 (1981). Leader
sequences have also been shown to influence
translation dramatically (Roberts et al., 1979 a, b
supra). Binding of the ribosome may also be affected
by the nucleotide sequence following the AUG, which
affects ribosome binding. (See, e.g., Taniguchi et
al., J. Mol. Biol. 118: 533 (1978).)

     Vectors for use in producing large quantities of
the recombinant adenovirus-derived proteins of this
invention, penton base and fiber, are designed for the
expression of proteins in bacteria, in mammalian cells
or in insect cells. For expression in bacterial E.
coli, the expression vectors are preferably utilized
in conjunction with bacterial "host" cells adapted for
the production of useful quantities of proteins or
polypeptides. Such vectors may include a procaryotic
replicon i.e., a nucleotide sequence having the
ability to direct autonomous replication and
maintenance of the recombinant DNA molecule
extra-chromosomally in a procaryotic host cell, such
as a bacterial host cell, transformed therewith. Such
replicons are well known in the art. In addition,
those embodiments that include a procaryotic replicon
may also include a gene whose expression confers a
selective advantage, such as drug resistance, to a
bacterial host transformed therewith. Typical
bacterial drug resistance genes are those that confer
resistance to ampicillin or tetracycline. Vectors

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typically also contain convenient restriction sites
for insertion of translatable nucleotide sequences.

    The procaryotic expression vectors also contain
promoters which can be used in the microbial organism
5   for expression of its own proteins. Those promoters
most commonly used include the beta-lactamase and
lactose promoter systems and the tryptophan promoter
system as described in the European Patent Application
No. 0125023.

10   . Exemplary procaryotic expression vectors include
the plasmids pUC8, pUC9, pUC18, pBR322, and pBR329
available from BioRad Laboratories (Richmond, CA), pPL
and pKK223 available from Pharmacia (Piscataway, NJ),
and pBS and M13mp19 (Stratagene, La Jolla, CA). Other
15   exemplary vectors include pCMU (Nilsson, et al., Cell
58: 707 (1989)). Other appropriate vectors may also
be synthesized, according to known methods; for
example, vectors pCMU/K^b and pCMUII used in various
applications herein are modifications of pCMUIV
20   (Nilsson, et al., supra).

    Mammalian expression vector systems are also
contemplated for the expression of recombinant penton
base and fiber proteins for use in this invention.
For controlling expression in mammalian cells,
25   viral-derived promoters are most commonly used. For
example, frequently used promoters include polyoma,
adenovirus type 2, and Simian Virus 40 (SV40). The
early and late promoters of SV40 virus are
particularly useful because both are obtained easily
30   from the virus as a fragment which also contains the
SV40 viral origin of replication. Smaller or larger
SV40 fragments may also be used, provided there is
included the approximately 250 base pair sequence
extending from the Hind III restriction site toward

the Bgl I site located in the viral origin of
replication. Also contemplated is using the promoter
sequences normally associated with the desired
sequence for expression, in this instance, adenovirus

5   2. Origins of replication may be provided either by
construction of the vector to include an exogenous
origin, such as may be derived from SV40 or other
viral sources such as polyoma and adenovirus or may be
provided by the host cell chromosomal replication

10 mechanism. The latter is sufficient for integration
of the expression vector in the host cell chromosome.
        The preferred expression vector system for use
preparing large quantities of recombinant penton base
and fiber proteins is the baculovirus expression

15 vector system, the details of which are described in
Example 1. It will be understood that this invention,
although described herein in terms of a preferred
embodiment, should not be construed as limited to the
host cells, expression vectors and expression vectors

20 systems exemplified. Other expression vector systems,
well known to one of ordinary skill in the art and
described by Kaufman et al., In "Current Protocols in
Molecular Biology", Ausubel et al., eds., Unit 16, New
York (1990), are contemplated for preparing

25 recombinant penton base and fiber proteins for use in
this invention.
        3.   Expression and Purification of Recombinant
              Penton Base and Fiber Proteins
        To express the recombinant penton base and

30 fiber proteins for use in this invention, the
expression vectors containing the nucleotide sequences
that encode the preferred proteins are transfected
into the recipient hosts cells described above. The
host cell can be either procaryotic or eucaryotic.

35 Bacterial cells are preferred procaryotic host cells

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and typically are a strain of *E. coli* such as the
MC1061 or JM109 strains. Preferred eucaryotic host
cells include yeast, insect and mammalian cells, where
the latter are those from mouse, rat, monkey or human
5   fibroblastic cell lines. Preferred eucaryotic host
cells include Chinese hamster ovary (CHO) cells
available from the ATCC as CCL61 and NIH Swiss mouse
embryo cells NIH/3T3 available from the ATCC ar
CRL1658. Most preferred for the expression of the
10 recombinant penton base and fiber proteins is an
insect cell, which include *Spodoptera frugiperda* and
Trichoplusia ni strains as described in Example 1.

Transfection may be accomplished via numerous
methods, depending on the type of vector used,
15 including the calcium phosphate method, the
DEAE-dextran method, the stable transfer method,
electroporation, or via the liposome mediation method.
Numerous texts are available which set forth known
transfection methods and other procedures for
20 introducing nucleotides into cells; see, e.g., Current
Protocols in Molecular Biology, John Wiley & Sons, NY
(1991). The preferred methods of transfection are
performed as described in Example 1.

Successfully transformed cells, i.e., cells that
25 contain the penton base or fiber expression vectors,
are usually monitored by an appropriate immunological,
functional or visual assay. For example, with the
baculovirus vector expression vector system
transfected into host insect cells, the screening for
30 successfully transfected cells is accomplished by
visual inspection in a plaque assay for the presence
or absence of occlusion bodies as described in Example
1. Wild-type viral plaques contain the occlusions
whereas recombinant transfected viral plaques lack
35 occlusions.

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     In addition to the direct assaying for the presence of recombinants, successful transformation can be confirmed by well known immunological methods when the recombinant DNA is capable of directing the expression of a protein of this invention. For example, samples of a culture containing cells suspected of being transformed are harvested and assayed for the subject expressed protein of this invention using either monoclonal or polyclonal antibodies that are specific for the expressed protein, such as anti-penton base or anti-fiber antibodies.

     Purification of the expressed recombinant penton base and fiber proteins is accomplished by a variety of techniques depending on the selected expression vector system. For the preferred baculovirus expression vector system for use in obtaining large quantities of recombinant penton base and fiber proteins, following the purification of recombinant virus and preparation of high titer viral stocks, the viral stock containing the recombinant vector is used to infect host insect cells as described in Example 1. The constitutively expressed recombinant proteins are then purified by lysing cells with hypotonic buffer and collecting the intracellular proteins by centrifugation.

     Purified recombinant penton base has a number of properties which make it ideal for gene therapy applications. For example, penton base is capable of saturable and specific binding to human epithelial cells. The affinity value is about $Kd = 55$ nM, or approximately 8.9×10^4 sites per cell. Binding occurs at sites of membrane contact with the cell substratum. Binding to epithelial cells is

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magnesium-dependent. Penton base binding to
epithelial cells is 5-10 fold greater than binding to
fibroblasts. Binding of penton base to epithelial
cells is increased 10-fold at low pH.

D.   Systems for the Expression of Therapeutic
      Nucleotide Sequences Following Gene Transfer
      Mediated by Adenovirus-derived Proteins
      1.   Nucleic Acid Segments
            A therapeutic nucleotide composition of the
present invention comprises a nucleotide sequence
encoding a therapeutic molecule as described in
Section F below. A therapeutic nucleotide composition
may further comprise an enhancer element or a promoter
located 5' to and controlling the expression of said
therapeutic nucleotide sequence or gene. The promoter
is a DNA segment that contains a DNA sequence that
controls the expression of a gene located 3' or
downstream of the promoter. The promoter is the DNA
sequence to which RNA polymerase specifically binds
and initiates RNA synthesis (transcription) of that
gene, typically located 3' of the promoter.
            The subject therapeutic nucleotide composition
consists of a nucleic acid molecule that comprises at
least 2 different operatively linked DNA segments.
The DNA can be manipulated and amplified by PCR as
described in Section C and by using the standard
techniques described in Molecular Cloning: A
Laboratory Manual, 2nd Edition, Maniatis et al., eds.,
Cold Spring Harbor, New York (1989). Typically, to
produce a therapeutic nucleotide composition of the
present invention, the sequence encoding the selected
therapeutic composition and the promoter or enhancer
are operatively linked to a vector DNA molecule
capable of autonomous replication in a cell either *in
vivo* or *in vitro*. By operatively linking the enhancer

element or promoter and the nucleotide sequence
encoding the therapeutic nucleotide composition to the
vector, the attached segments are replicated along
with the vector sequences. Thus, a recombinant DNA

5   molecule (rDNA) of the present invention is a hybrid
DNA molecule comprising at least 2 nucleotide
sequences not normally found together in nature.
    The therapeutic nucleotide composition of the
present invention is from about 20 base pairs to about

10 100,000 base pairs in length. Preferably the nucleic
acid molecule is from about 50 base pairs to about
50,000 base pairs in length. More preferably the
nucleic acid molecule is from about 50 base pairs to
about 10,000 base pairs in length. Most preferred is

15 a nucleic acid molecule from about 50 pairs to about
4,000 base pairs in length. The therapeutic
nucleotide can be a gene or gene fragment that encodes
a protein or peptide that provides the desired
therapeutic effect such as replacement of alpha

20 1-antitrypsin or cystic fibrosis transmembrane
regulator protein and the like. Alternatively, the
therapeutic nucleotide can be a DNA or RNA
oligonucleotide sequence that exhibits enzymatic
therapeutic activity. Examples of the latter include

25 antisense oligonucleotides that will inhibit the
transcription of deleterious genes or ribozymes that
act as site-specific ribonucleases for cleaving
selected mutated gene sequences. In another
variation, a therapeutic nucleotide sequence of the

30 present invention may comprise a DNA construct capable
of generating therapeutic nucleotide molecules,
including ribozymes and antisense DNA, in high copy
numbers in target cells, as described in published PCT
application No. WO 92/06693 (the disclosure of which

35 is incorporated herein by reference).

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A regulatable promoter is a promoter where the
rate of RNA polymerase binding and initiation is
modulated by external stimuli. Such stimuli include
compositions light, heat, stress and the like.
5   Inducible, suppressible and repressible promoters are
regulatable promoters. Regulatable promoters may also
include tissue specific promoters. Tissue specific
promoters direct the expression of that gene to a
specific cell type. Tissue specific promoters cause
10   the gene located 3' of it to be expressed
predominantly, if not exclusively in the specific
cells where the promoter expressed its endogenous
gene. Typically, it appears that if a tissue-specific
promoter expresses the gene located 3' of it at all,
15   then it is expressed appropriately in the correct cell
types as has been reviewed by Palmiter et al., _Ann._
Rev. Genet. 20: 465-499 (1986).

When a tissue specific promoter controls the
expression of a gene, that gene will be expressed in a
20   small number of tissues or cell types rather than in
substantially all tissues and cell types. Examples of
tissue specific promoters include the immunoglobulin
promoter described by Brinster et al., _Nature 306_:
332-336 (1983) and Storb et al., _Nature 310_: 238-231
25   (1984); the elastase-I promoter described by Swift et
al., _Cell 38_: 639-646 (1984); the globin promoter
described by Townes et al., _Mol. Cell. Biol. 5_:
1977-1983 (1985), and Magram et al., _Mol. Cell. Biol._
_9_: 4581-4584 (1989), the insulin promoter described by
30   Bucchini et al., _PNAS USA. 83_: 2511-2515 (1986) and
Edwards et al., _Cell 58_: 161 (1989); the
immunoglobulin promoter described by Ruscon et al.,
Nature 314: 330-334 (1985) and Grosscheld et al., _Cell_
_38_: 647-658 (1984); the alpha actin promoter described
35   by Shani, _Mol. Cell. Biol. 6_: 2624-2631 (1986); the

alpha crystalline promoter described by Overbeek et
al., _PNAS USA 82_: 7815-7819 (1985); the prolactin
promoter described by Crenshaw et al., _Genes and
Development 3_: 959-972 (1989); the proopiomelanocortin
5   promoter described by Tremblay et al., _PNAS USA 85_:
8890-8894 (1988); the beta-thyroid stimulating hormone
(BTSH) promoter described by Tatsumi et al., _Nippon
Rinsho 47_: 2213-2220 (1989); the mouse mammary tumor
virus (MMTV) promoter described by Muller et al., _Cell_
10   _54_: 105 (1988); the albumin promoter described by
Palmiter et al., _Ann. Rev. Genet. 20_: 465-499 (1986);
the keratin promoter described by Vassar et al., _PNAS_
USA 86: 8565-8569 (1989); the osteonectin promoter
described by McVey et al., _J. Biol. Chem. 263_:
15   11,111-11,116 (1988); the prostate-specific promoter
described by Allison et al., _Mol. Cell. Biol. 9_:
2254-2257 (1989); the opsin promoter described by
Nathans et al., _PNAS USA 81_: 4851-4855 (1984); the
olfactory marker protein promoter described by
20   Danciger et al., _PNAS USA 86_: 8565-8569 (1989); the
neuron-specific enolase (NSE) promoter described by
Forss-Pelter et al., _J. Neurosci. Res. 16_: 141-151
(1986); the L-7 promoter described by Sutcliffe,
Trends in Genetics 3: 73-76 (1987) and the protamine 1
25   promoter described Peschon et al., _Ann. New York Acad._
Sci. 564: 186-197 (1989) and Braun et al., _Genes and_
Development 3: 793-802 (1989).

        2.   _Expression Vector Systems_
              The introduction of exogenous DNA into
30   eucaryotic cells has become one of the most powerful
tools of the molecular biologist. The term
"exogenous" encompasses any therapeutic composition of
this invention which is administered by the
therapeutic methods of this invention. Thus,
35   "exogenous" is also referred to as "foreign,

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nonnative, and the like". The methods of this
invention requires efficient delivery of the DNA into
the nucleus of the recipient cell and subsequent
identification of cells that are expressing the
5   foreign DNA.

Engineered vectors such as plasmids or
bacteriophages (phages) or other DNA sequences that
are able to replicate in a host cell can be used to
construct cells that act as factories to produce large
10   amounts of specific viral proteins. Recombinant
plasmids will be used herein as exemplary vectors,
also called cloning vehicles. See U.S. Patent No.
4,338,397, incorporated herein by reference.

Plasmids are extrachromosomal genetic elements
15   found in a variety of bacterial species. They are
typically double-stranded, closed, circular DNA
molecules. A widely-used plasmid is pBR322, a vector
whose nucleotide sequence and endonuclease cleavage
sites are well known.

20   Nucleic acid production using plasmid or phage
vectors has become very straightforward. The plasmid
or phage DNA is cleaved with a restriction
endonuclease and joined *in vivo* to a foreign DNA of
choice. The resulting recombinant plasmid or phage is
25   then introduced into a cell such as *E. coli*, and the
cell so produced is induced to produce many copies of
the engineered vector. Once a sufficient quantity of
DNA is produced by the cloning vector, the produced
foreign DNA is excised and placed into a second vector
30   to produce or transcribe the protein or polypeptide
encoded by the foreign gene.

Depending on the DNA (intact gene, cDNA, or
bacterial gene), it may be necessary to provide
eucaryotic transcription and translation signals to
35   direct expression in recipient cells either *in vivo* or

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in vitro. These signals may be provided by combining
the foreign DNA in vitro with an expression vector.

Expression vectors contain sequences of DNA that
are required for the transcription of cloned genes and
the translation of their messenger RNA's (mRNA's) into
proteins. Typically, such required sequences or
control elements are: (1) a promoter that signals the
starting point for transcription; (2) a terminator
that signals the ending point of transcription; (3) an
operator that regulates the promotor; (4) a ribosome
binding site for the initial binding of the cells'
protein synthesis machinery; and (5) start and stop
codons that signal the beginning and ending of protein
synthesis.

To be useful, an expression vector should possess
several additional properties. It should be
relatively small and contain a strong promoter. The
expression vector should carry one or more selectable
markers to allow identification of transformants. It
should also contain a recognition site for one or more
restriction enzymes in regions of the vector that are
not essential for expression.

The construction of expression vectors is,
therefore, a complicated and somewhat unpredictable
venture. The only true test of the effectiveness of
an expression vector is to measure the frequency with
which the synthesis of the appropriate mRNA is
initiated. However, quantitation of mRNA is tedious,
and it is often difficult to obtain accurate
measurements. Other more practicable means have,
therefore, been developed to detect transformation.

One such means has been to monitor synthesis of
foreign proteins in transformed cells with enzymatic
assays. Several marker genes have been developed for
indicating that transformation has occurred.

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Another means used to monitor transformation
involves the use of immunological reagents. If the
level of expressed protein is sufficiently high, then
cytoplasmic or surface immunofluorescence with an
5 antibody conjugated to a fluorescent dye such as
fluorescein or rhodamine may be used to detect
vector-specific protein expression products.

More commonly, transformed cells are cultured in
the presence of radioactivity after
10 immunoprecipitation. This approach has used
Staphylococcus aureus protein A selection of immune
complexes (Kessler, J. Immunol. 115: 1617-1624 (1975))
and the Western blotting procedure (Renart et al.,
PNAS USA 76: 3116-3120 (1979)) to detect
15 transformation-specific markers.

A vector of the present invention is a nucleic
acid (preferably DNA) molecule capable of autonomous
replication in a cell and to which a DNA segment,
e.g., gene or polynucleotide, can be operatively
20 linked so as to bring about replication of the
attached segment. In the present invention, one of
the nucleotide segments to be operatively linked to
vector sequences encodes at least a portion of a
therapeutic molecule. Preferably, the entire
25 peptide-coding sequence of the therapeutic gene is
inserted into the vector and expressed; however, it is
also feasible to construct a vector which also
includes some non-coding sequences as well.
Preferably, the non-coding sequences are excluded.
30 Alternatively, a nucleotide sequence for a soluble
form of a polypeptide may be utilized. Another
preferred vector includes a nucleotide sequence
encoding at least a portion of a therapeutic
nucleotide sequence operatively linked to the vector
35 for expression.

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As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. Preferred vectors are those capable of autonomous replication and expression of structural gene products present in the nucleotide (DNA) segments to which they are operatively linked.

As used herein with regard to DNA sequences or segments, the phrase "operatively linked" means the sequences or segments have been covalently joined into one piece of DNA, whether in single or double stranded form.

The choice of vector into which a therapeutic nucleotide sequence of this invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., vector replication and protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules.

In various embodiments, a vector is utilized for the production of therapeutic proteins or polypeptides useful in the present invention. Such vectors are preferably utilized in conjunction with eucaryotic cells. Such vectors may include a eucaryotic replicon i.e., a nucleotide sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally or stably in a eucaryotic recipient cell. Such replicons are well known in the art. In addition, those embodiments that include a eucaryotic replicon may also include a gene whose expression confers a selective advantage, such as drug resistance. Vectors typically also contain convenient restriction sites for insertion of translatable nucleotide sequences. Exemplary vectors

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include the Chinese hamster ovary cell expression
vectors, vaccinia virus expression system, vaccinia
virus/T7 RNA polymerase hybrid system and the like as
described by Kaufman et al., In "Current Protocols in
5   Molecular Biology", Ausubel et al., eds., Unit
16.12.5, New York (1990).

    A sequence of nucleotides adapted for directional
ligation, i.e., a polylinker, is a region of the
expression vector that (1) operatively links for
10   replication and transport the upstream and downstream
nucleotide sequences and (2) provides a site or means
for directional ligation of a nucleotide sequence into
the vector. Typically, a directional polylinker is a
sequence of nucleotides that defines two or more
15   restriction endonuclease recognition sequences, or
restriction sites. Upon restriction cleavage, the two
sites yield cohesive termini to which a translatable
nucleotide sequence can be ligated to the expression
vector. Preferably, the two restriction sites
20   provide, upon restriction cleavage, cohesive termini
that are non-complementary and thereby permit
directional insertion of a translatable nucleotide
sequence into the vector. In one embodiment, the
directional ligation means is provided by nucleotides
25   present in the upstream nucleotide sequence,
downstream nucleotide sequence, or both. In another
embodiment, the sequence of nucleotides adapted for
directional ligation comprises a sequence of
nucleotides that defines multiple directional cloning
30   means. Where the sequence of nucleotides adapted for
directional ligation defines numerous restriction
sites, it is referred to as a multiple cloning site.

    A translatable nucleotide sequence is a linear
series of nucleotides that provide an uninterrupted
35   series of at least 8 codons that encode a polypeptide

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in one reading frame. Preferably, the nucleotide
sequence is a DNA sequence. The vector itself may be
of any suitable type, such as a viral vector (RNA or
DNA), naked straight-chain or circular DNA, or a
5   vesicle or envelope containing the nucleic acid
material and any polypeptides that are to be inserted
into the cell. With respect to vesicles, techniques
for construction of lipid vesicles, such as liposomes
described in Section E, are well known. Such
10   liposomes may be targeted to particular cells using
other conventional techniques, such as providing an
antibody or other specific binding molecule on the
exterior of the liposome. See, e.g., A. Huang, et
al., J. Biol. Chem. 255: 8015-8018 (1980).

15   Most useful vectors contain multiple elements
including one or more of the following, depending on
the nature of the recipient cell: an SV40 origin of
replication for amplification to high copy number; an
efficient promoter element for high-level
20   transcription initiation; mRNA processing signals such
as mRNA cleavage and polyadenylation sequences (and
frequently, intervening sequences as well);
polylinkers containing multiple restriction
endonuclease sites for insertion of foreign DNA;
25   selectable markers that can be used to select cells
that have stably integrated the plasmid DNA; and
plasmid replication control sequences to permit
propagation in bacterial cells. In addition to the
above, many vectors also contain an inducible
30   expression system that is regulated by an external
stimulus. Sequences from a number of promoters that
are required for induced transcription have been
identified and engineered into expression vectors to
obtain inducible expression. Several useful inducible
35   vectors have been based on induction by γ-interferon,

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heat-shock, heavy metal ions, and steroids (e.g.
glucocorticoids). (See, e.g., Kaufman, Meth. Enzymol.
185: 487-511 (1990).) Other promoters contemplated
for use in this invention are described in Example 5.

5   A preferred vector in which therapeutic
nucleotide compositions of this invention are present
is a plasmid; more preferably, it is a
high-copy-number plasmid. It is also desirable that
the vector contain an inducible promoter sequence, as
10   inducible promoters tend to limit selection pressure
against cells into which such vectors (which are often
constructed to carry non-native or chimeric nucleotide
sequences) have been introduced by the
adenovirus-derived proteins of this invention. It is
15   also preferable that the vector of choice be best
suited for expression in the preselected recipient
cell type depending on the nature of the gene
replacement therapy.

        A tissue containing a therapeutic nucleotide
20   sequence of the present invention may also be produced
by directly introducing the vector containing the
sequence into an animal or by linking the therapeutic
oligonucleotide directly to the adenovirus-derived
proteins. Direct vector delivery in vivo may be
25   accomplished by transducing the desired cells and
tissues with viral vectors or other physical gene
transfer vehicles. Other physical agents including
naked plasmids, cloned genes encapsulated in
targetable liposomes (see Section E below) or in
30   erythrocyte ghosts have been use to introduce genes,
proteins, toxins and other agents directly into whole
animals.

        Direct injection of therapeutic nucleotide
sequences, with or without penton -- but preferably in
35   conjunction therewith -- is also a viable alternative

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for delivery of therapeutic sequences. For example,
the injection of naked calcium phosphate-precipitated
plasmid into rat liver and rat spleen or a
protein-coated plasmid into the portal vein has
5   resulted in gene expression of the foreign gene the
rat livers. (See, e.g., Kaneda, et al., _Science_,
243:375 (1989), incorporated by reference herein.)

E.   Liposomes

In preferred embodiments, a subject therapeutic
10   sequence, penton base, fiber or penton complex, penton
conjugates and compositions of the present invention
(hereinafter, "active ingredient") is covalently
linked to a carrier, such as a phospholipid. More
preferably, the active ingredient is removably
15   inserted into a liposome, i.e., incorporated
(anchored) into the liposome bilayer via the LDL
binding moiety. (See, e.g., Gregoriadis, _Trends in
Biotech.__, 3:235-241 (1985) and Eriksson et al., in
Liposome Technology Vol. II, G. Gregoriadis (ed.), CRC
20   Press, Boca Raton, FL, pp. 141-156. The disclosures
of these articles are incorporated herein by
reference.) Since the penton base does not typically
include a transmembrane anchor sequence, it is
necessary to construct a lipid binding site on the
25   virus protein in order that it may associate with
liposomes.

Therapeutic nucleotide sequences and compositions
according to the present invention can be administered
in a liposome (micelle) formulation which can be
30   administered by application to mucous membranes of
body cavities. Juliano et al., _J. Pharmacol. Exp._
Ther. 214: 381 (1980). Liposomes are prepared by a
variety of techniques well known to those skilled in
the art to yield several different physical
35   structures, ranging from the smallest unilamellar

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vesicles of approximately 20 to 50 nanometers in
diameter up to multilamellar vesicles of tens of
microns in diameter. Gregoriadis (ed.), _Liposome_
Technology 1, CRC Press (1984). Therapeutic
nucleotide sequences and compositions for nasal
formulations are preferably hydrated with a
lyophilized powder of multilamellar vesicles to form
liposomes containing the sequences and compositions
according to the present invention.

Therapeutic sequences, penton base, fiber or
penton complex, penton conjugates and compositions of
the present invention (hereinafter, "active
ingredient") may also be incorporated into liposomal
vesicles via reverse loading (see U.S. Pat. No.
5,104,661), or in the manner described for the
incorporation of amphotericin B into lipid vesicles.
(See, e.g., Lopez-Berenstein, et al., _J. Infect. Dis._
_151_: 704-710 (1985); Lopez-Berenstein, _Antimicrob._
Agents Chemother. 31: 675-8 (1987); Lopez-Berenstein,
et al., _J. Infect. Dis. 150_: 278-283 (1984); and
Mehta, et al., _Biochem. Biophys. Acta 770_: 230-4
(1984).) Delivery may also be accomplished using
liposomes with enhanced circulation time (see U.S.
Pat. No. 5,013,556). Use of liposomes to deliver
therapeutic nucleotide sequences according to the
present invention may also be utilized to regulate
expression of target sequences or to limit the
proliferation of virus or retrovirus. (See, e.g.,
published PCT application No. WO 92/06192,
incorporated herein by reference.)

The amount of active ingredient incorporated into
liposomes may be about 0.1 μg active ingredient per mg
lipid to about 1 mg per mg lipid. The dosage amount
of active ingredient administered in lipid
encapsulated form is preferably about 0.1-1mg per mg

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lipid.

Direct vector delivery *in vivo* may be
accomplished via liposomal delivery. Various physical
agents including therapeutic nucleotide sequences
5   encapsulated in targetable liposomes have been use to
introduce genes, proteins, toxins and other agents
directly into whole animals. See, for example, the
liposome-mediated gene delivery *in vivo* and expression
of preproinsulin genes in recipient rats described by
10   Nicolaou, et al., PNAS USA 80:1068 (1983) and Soriano,
et al., PNAS USA 80:7128 (1983). All cited
disclosures are incorporated herein by reference.

F.   Therapeutic Sequences and Compositions

In various alternative embodiments of the present
15   invention, therapeutic sequences and compositions
useful for practicing the therapeutic methods
described herein are contemplated. Therapeutic
compositions of the present invention may contain a
physiologically tolerable carrier together with one or
20   more therapeutic nucleotide sequences of this
invention, dissolved or dispersed therein as an active
ingredient. In a preferred embodiment, the
composition is not immunogenic or otherwise able to
cause undesirable side effects when administered to a
25   mammal or human patient for therapeutic purposes.

As used herein, the terms "pharmaceutically
acceptable", "physiologically tolerable" and
grammatical variations thereof, as they refer to
compositions, carriers, diluents and reagents, are
30   used interchangeably and represent that the materials
are capable of administration to or upon a mammal
without the production of undesirable physiological
effects such as nausea, dizziness, gastric upset and
the like.

35   For example, the present invention comprises

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therapeutic compositions useful in the specific
targeting of epithelial or non-epithelial cells as
well as in delivering a therapeutic nucleotide
sequence to those cells. Therapeutic compositions
5   designed to preferentially target to epithelial cells
may comprise an adenovirus-derived protein and a
therapeutic nucleotide sequence. As described
hereinabove, penton base or penton complex are
preferred adenovirus-derived proteins useful in the
10   presently-disclosed therapeutic compositions and
methods.

        Compositions designed to preferentially target
non-epithelial cells may include an adenovirus-derived
protein-ligand conjugate and a therapeutic nucleotide
15   sequence. Examples of useful ligands directed to
specific receptors (identified in parentheses) include
the V3 loop of HIV gp120 (CD4); transferrin
(transferrin receptor); LDL (LDL receptors); and
deglycosylated proteins (asialoglycoprotein receptor).
20   Polypeptides having a sequence that includes an amino
acid residue sequence selected from the group
comprising -EDPGFFNVE- (SEQ ID NO 6) and -EDPGKQLYNVE-
(SEQ ID NO 7) are capable of targeting receptors such
as the CR2 receptor, and are thus useful in
25   compositions disclosed herein.

        Useful ligands also include antibodies and
attachment sequences, as well as receptors themselves.
Antibodies to cell receptor molecules such as
integrins and the like, MHC Class I and Class II,
30   asialoglycoprotein receptor, transferrin receptors,
LDL receptors, CD4, and CR2 are but a few useful
according to the present invention. It is also
understood that the ligands typically bound by
receptors, as well as analogs to those ligands, may be
35   used as cellular targeting agents as disclosed herein.

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Exemplary and preferred nucleotide sequences
encode an expressible peptide, polypeptide or protein,
and may further include an active constitutive or
inducible promoter sequence. For example, preferred

5   therapeutic nucleotide sequences according to the
present invention are capable of delivering HIV
antisense nucleotides to latently-infected T cells via
CD4. Similarly, delivery of Epstein-Barr Virus (EBV)
EBNa-1 antisense nucleotides to B cells via CR2 is

10   capable of effecting therapeutic results.

The preparation of a pharmacological composition
that contains active ingredients dissolved or
dispersed therein is well understood in the art.
Typically such compositions are prepared as

15   injectables either as liquid solutions or suspensions,
however, solid forms suitable for solution, or
suspensions, in liquid prior to use can also be
prepared. The preparation can also be emulsified, or
formulated into suppositories, ointments, creams,

20   dermal patches, or the like, depending on the desired
route of administration.

The active ingredient can be mixed with
excipients which are pharmaceutically acceptable and
compatible with the active ingredient and in amounts

25   suitable for use in the therapeutic methods described
herein. Suitable excipients are, for example, water,
saline, dextrose, glycerol, ethanol or the like and
combinations thereof, including vegetable oils,
propylene glycol, polyethylene glycol and benzyl

30   alcohol (for injection or liquid preparations); and
vaseline, vegetable oil, animal fat and polyethylene
glycol (for externally applicable preparations). In
addition, if desired, the composition can contain
wetting or emulsifying agents, isotonic agents,

35   dissolution promoting agents, stabilizers, colorants,

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antiseptic agents, soothing agents and the like
additives (as usual auxiliary additives to
pharmaceutical preparations), pH buffering agents and
the like which enhance the effectiveness of the active
5   ingredient.

The therapeutic compositions of the present
invention can include pharmaceutically acceptable
salts of the components therein. Pharmaceutically
acceptable salts include the acid addition salts
10   (formed with the free amino groups of the polypeptide)
that are formed with inorganic acids such as, for
example, hydrochloric or phosphoric acids, or such
organic acids as acetic, tartaric, mandelic and the
like. Salts formed with the free carboxyl groups can
15   also be derived from inorganic bases such as, for
example, sodium, potassium, ammonium, calcium or
ferric hydroxides, and such organic bases as
isopropylamine, trimethylamine, 2-ethylamino ethanol,
histidine, procaine and the like.
20   Physiologically tolerable carriers are well known
in the art. Exemplary of liquid carriers are sterile
aqueous solutions that contain no materials in
addition to the active ingredients and water, or
contain a buffer such as sodium phosphate at
25   physiological pH value, physiological saline or both,
such as phosphate-buffered saline. Still further,
aqueous carriers can contain more than one buffer
salt, as well as salts such as sodium and potassium
chlorides, dextrose, polyethylene glycol and other
30   solutes.

Liquid compositions can also contain liquid
phases in addition to and to the exclusion of water.
Exemplary of such additional liquid phases are
glycerin, vegetable oils such as cottonseed oil, and
35   water-oil emulsions.

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A therapeutic composition typically contains an amount of a therapeutic nucleotide sequence of the present invention sufficient to deliver a therapeutically effective amount to the target tissue, typically an amount of at least 0.1 weight percent to about 90 weight percent of therapeutic nucleotide sequence per weight of total therapeutic composition. A weight percent is a ratio by weight of therapeutic nucleotide sequence to total composition. Thus, for example, 0.1 weight percent is 0.1 grams of DNA segment per 100 grams of total composition.

The therapeutic nucleotide compositions comprising synthetic oligonucleotide sequences of the present invention can be prepared using any suitable method, such as, the phosphotriester or phosphodiester methods. See Narang et al., Meth. Enzymol., 68:90, (1979); U.S. Patent No. 4,356,270; and Brown et al., Meth. Enzymol., 68:109, (1979).

For therapeutic oligonucleotides sequence compositions in which a family of variants is preferred, the synthesis of the family members can be conducted simultaneously in a single reaction vessel, or can be synthesized independently and later admixed in preselected molar ratios.

For simultaneous synthesis, the nucleotide residues that are conserved at preselected positions of the sequence of the family member can be introduced in a chemical synthesis protocol simultaneously to the variants by the addition of a single preselected nucleotide precursor to the solid phase oligonucleotide reaction admixture when that position number of the oligonucleotide is being chemically added to the growing oligonucleotide polymer. The addition of nucleotide residues to those positions in the sequence that vary can be introduced

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simultaneously by the addition of amounts, preferably
equimolar amounts, of multiple preselected nucleotide
precursors to the solid phase oligonucleotide reaction
admixture during chemical synthesis. For example,

5   where all four possible natural nucleotides (A,T,G and
C) are to be added at a preselected position, their
precursors are added to the oligonucleotide synthesis
reaction at that step to simultaneously form four
variants.

10   This manner of simultaneous synthesis of a family
of related oligonucleotides has been previously
described for the preparation of "degenerate
oligonucleotides" by Ausubel et al, in "Current
Protocols in Molecular Biology", Suppl. 8. p.2.11.7,

15   John Wiley & Sons, Inc., New York (1991), and can
readily be applied to the preparation of the
therapeutic oligonucleotide compositions described
herein.

           Nucleotide bases other than the common four

20   nucleotides (A,T,G or C), or the RNA equivalent
nucleotide uracil (U), can be used in the present
invention. For example, it is well known that inosine
(I) is capable of hybridizing with A, T and G, but not
C.

25   Thus, where all four common nucleotides are to
occupy a single position of a family of
oligonucleotides, that is, where the preselected
therapeutic nucleotide composition is designed to
contain oligonucleotides that can hybridize to four

30   sequences that vary at one position, several different
oligonucleotide structures are contemplated. The
composition can contain four members, where a
preselected position contains A,T,G or C.
Alternatively, the composition can contain two

35   members, where a preselected position contains I or C,

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and has the capacity the hybridize at that position to
all four possible common nucleotides. Finally, other
nucleotides may be included at the preselected
position that have the capacity to hybridize in a
5   non-destabilizing manner with more than one of the
common nucleotides in a manner similar to inosine.
G.   Therapeutic Methods
      Various therapeutic methods are also contemplated
by the present invention. For example, it has now
10   been discovered that proteins derived from adenovirus
are capable of delivering a therapeutic nucleotide
sequence to a specific cell or tissue, thereby
expanding and enhancing treatment options available in
numerous conditions in which more conventional
15   therapies are of limited efficacy.
      The therapeutic nucleotide sequences described
herein and compositions including same have a number
of uses, and may be used in vitro or in vivo. For
example, the compositions may be used prophylactically
20   or therapeutically in vivo to disrupt HIV infection
and mechanisms of action by inhibiting gene expression
or activation, via delivery of antisense HIV sequences
or ribozymes to T cells or monocytes. Other useful
therapeutic nucleotide sequences include antisense
25   nucleotide sequences complementary to EBV EBNa-1 gene.
Use of such therapeutic sequences may remediate or
prevent latent infection of B cells with EBV. (As
discussed in Example 5 below, targeting and delivery
may be accomplished via the use of various ligands,
30   receptors, and other appropriate targeting agents.)
      The method comprises, in one embodiment,
contacting human cells infected with EBV or HIV with a
therapeutically effective amount of a pharmaceutically
acceptable composition comprising a therapeutic
35   nucleotide sequence of this invention. In a related

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embodiment, the contacting involves introducing the
therapeutic nucleotide sequence composition into cells
having an EBV or HIV-mediated infection.

   The present invention also contemplates methods
5   for determining the efficacy of the within-disclosed
therapeutic compositions and methods. One such method
for confirming efficacy utilizes the human/SCID
(severe combined immunodeficient) mouse model of EBV-
induced LPD (lymphoproliferative disease) to ascertain
10   whether EBV-antisense therapeutic nucleotide sequences
block tumor formation.

   For example, it has been observed that SCID/hu
chimeric mice resulting from SCID mice reconstituted
with human lymphocytes from EBV positive donors
15   develop aggressive human B-cell tumors containing
viral DNA, or fatal LPD of human B-cell origin
containing EBV DNA. (See, e.g., Pisa, et al., Blood
79: 173-179 (1992); Rowe, et al., Curr. Top.
Microbiol. Immunol. 166: 325 (1990); and Cannon, et
20   al., J. Clin. Invest. 85: 1333-1337 (1990)).
Therefore, one means of assessing the ability of
therapeutic nucleotide sequences to block EBV-
associated tumor formation may be described
essentially as follows. SCID mice are injected with
25   lymphocytes from peripheral blood (PBL) or palatine
tonsils (lymph nodes, LN) from EBV-seronegative
individuals. The SCID/hu mice are then infected with
EBV about 7 days thereafter. Administration of
therapeutic nucleotide sequences may proceed prior to,
30   concurrent with, or shortly after the EBV infection.
Since EBV-infected SCID/hu mice tend to develop
clinical signs of tumor 1-2 months after EBV
infection, the mice are monitored over this period of
time to ascertain the effect of the therapeutic
35   nucleotide sequences and compositions on the course of

the infection and tumor (or LPD) progression.
Alternatively, the SCID/hu mice may be monitored to
ascertain whether circulating cells with human and EBV
DNA are present, as these cells tend to appear before
5   the appearance of overt tumors (Pisa, et al., _Blood_
_79_: 173-179 (1992).)

        It is expressly to be understood that the above
is an example only, as it is one of several methods
available for determining the effectiveness of the
10 within-described nucleotide sequences, compositions
and therapeutic methods.

                        EXAMPLES

        The following examples are intended to
15 illustrate, but not limit, the present invention.

                        Example 1

            Cloning, Expression and Purification of
            Adenovirus Type 2 Penton Base and Fiber

        For the preparation of recombinant penton base
20 and fiber from human adenovirus type 2 (hereinafter
referred to as Ad2), PCR (polymerase chain reaction)
amplification was performed on DNA extracted from the
virus following propagation in HeLa cells as described
by Horwitz, "Adenoviridae and their replication", in
25 _Virology_, Fields and Knipe, eds., Raven Press, NY
(1990) pp. 1679-1740. Briefly, HeLa cells (American
Type Culture Collection, Rockville, MD (ATCC accession
number CCL 2)), maintained in Eagle's MEM with
non-essential amino acids and 90% Earle's BSS
30 containing 10% fetal bovine serum, were infected with
Ad2 obtained from ATCC having ATCC accession number
VR-846 having a multiplicity of infection (MOI) of 10.
Virus infected cells were then harvested 2-3 days
later by pelleting by centrifugation the cells at
35 10,000 rpm for 10 minutes at 4°C. The harvested cells

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were freeze-thawed 5 times to release intracellular
particles and then the cell debris was removed by
centrifugation. The resulting cell lysate was
subjected to density gradient ultracentrifugation on
cesium chloride ($CsCl_2$) gradients (25-40%). The virus
band was removed and dialyzed against PBS. Virus
particle concentration was determined by the Bradford
assay using a molecular weight of 1.7×10^8 Daltons
for adenovirus.

DNA was extracted from the propagated and
purified Ad2 stocks by admixing the virus with 200
micrograms (μg) of proteinase K at a concentration of
10 micrograms/milliliter (μg/ml) and maintained at 37
degrees Celsius (37°C) for 6 hours. The admixture was
then boiled for 15 minutes followed by 2 extractions
with phenol/chloroform. The extracted viral DNA was
then precipitated with ethanol and used as a template
for PCR amplification as described below.

PCR was then performed on the Ad2 template DNA to
form both amplified penton base and fiber. The 5' and
3' primers used to amplify the penton base were,
respectively, 5'-TTTCTAGAAGTATGCAGCGCGCG-3' (SEQ ID NO
2) and 5'-TTTCTAGAGATCAAAAAGTGCGGCT-3' (SEQ ID NO 3).
The underlined nucleotides indicate the nucleotide
sequence for incorporating the restriction enzyme site
Xba I into the amplified penton base nucleotide
sequence for subsequent cloning into the compatible
Nhe I restriction site in the 14 kilobase (kb)
baculovirus expression vector, pBlueBac II
(Invitrogen, San Diego, CA).

The 5' and 3' primers used to amplify the fiber
were, respectively, 5'-AAAGGATCCAGCTGATGAAACGCGCCA-3'
(SEQ ID NO 4) and 5'-TTTGGTACCAGCTGTTATTCCTGGGCA-3'
(SEQ ID NO 5). The first six underlined nucleotides

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(GGATCC) in the 5' oligonucleotide (SEQ ID NO 4)
indicate the nucleotide sequence for incorporating the
restriction enzyme site Bam HI into the amplified
fiber nucleotide sequence. The second six underlined

5   nucleotides (CAGCTG) with a C nucleotide overlap in
the 5' oligonucleotide indicate the nucleotide
sequence for incorporating the restriction enzyme site
Pvu II into the amplified fiber nucleotide sequence.
The 5' end of the 3' oligonucleotide (SEQ ID NO 5)

10   contained a Kpn I (GGTACC) restriction site (the first
six nucleotides). A Pvu II restriction site (CAGCTG)
with a C nucleotide overlap was also incorporated into
the 3' oligonucleotide as indicated by the underlined
sequence. The restriction sites identified herein

15   were amplified into the 5' and 3' ends of the fiber
PCR product for cloning the resultant into the
pBlueBac vector as described below.

        The penton base and fiber nucleotide sequences
were separately amplified by PCR in reactions

20   containing 85 microliters (μl) water, 10 μl PCR 10X
buffer (100 millimolar (mM) Tris-HCl at pH 8.0, 500 mM
KCl, 15 mM MgCl$_2$ and 0.1% (w/v) gelatin), 1 μl each of
the 5' and 3' oligonucleotide primers listed above for
a final concentration of 50 picomolar/μl, 1 μl 100X

25   dNTP's (final concentration of 200 micromolar (μM)
each), 1 μl Thermus aquaticus (Taq) polymerase
(Perkins-Elmer Corp., Norwalk, CT), and 10^9 plaque
forming units of purified viral DNA prepared above.
Thirty amplification cycles were performed with one

30   cycle consisting of 2 minutes at 72°C, 10 minutes at
94°C, 1 minute at 95°C, 1 minute at 60°C and 2 minutes
and 15 seconds at 72°C. The PCR products were
separately analyzed by gel electrophoresis to confirm
that the appropriately sized DNA was amplified. For

the penton base, the PCR product contained 1713 base
pairs (bp) for encoding the penton base bordered by
Xba I restriction site nucleotides. The nucleotide
sequence for the penton base gene of human adenovirus
type 2 has been described by Neumann et al., _Gene_
_69_:153-157 (1988). The fiber PCR product contained
1746 bp for encoding the fiber bordered by Bam HI/Pvu
II and Pvu II/Kpn I restriction site nucleotides. The
nucleotide sequence for the fiber gene of human
adenovirus type 2 has been described by Herisse et
al., _Nuc. Acids Res. 9_:4023-4041 (1981).

The resultant PCR products were purified and
separately ligated into pBlueBac II baculovirus
expression vector in the MaxBac® purchased from
Invitrogen (San Diego, CA). The penton base having
Xba I restriction sites on both the 5' and 3' ends was
first digested with Xba I then ligated into the
pBlueBac II vector previously digested with Nhe I to
form a recombinant pBlueBac II penton base
DNA-containing vector. Nhe I and Xba I are compatible
restriction sites thereby providing for ligation of
the PCR product into the expression vector. The fiber
PCR product was first digested with Bam HI and Kpn I
and directionally ligated into a similarly digested
pBluescript vector (Stratagene, La Jolla, CA). The
fiber nucleotide sequence was then removed from
pBluescript with the blunt end cutter, Pvu II, after
which Nhe I adapter oligonucleotides (Invitrogen) were
ligated to the isolated fiber sequence. The resultant
fiber nucleotide sequence having Nhe I ends was then
ligated into the Nhe I digested pBlueBac II vector to
form a recombinant pBlueBac II fiber DNA-containing
vector.

The baculovirus expression vector is a
helper-independent recombinant virus vector system

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used for expressing genes from sources including
bacteria, viruses, plants and mammals. Recombinant
fusion or nonfusion proteins have been reported to be
expressed at levels ranging from 1-500 mg/liter.

5   The pBlueBac II expression vector was derived
from the pJVNhe I expression vector described by
Vialard et al., _J. Virol. 64_:37-50 (1990). As the
pBlueBac II encodes ß-galactosidase, the screening of
recombinant baculovirus is simplified thereby not

10   requiring cotransfection with a selectable
marker-based approach. The vector has a unique Nhe I
restriction cloning site and the 5' polyhedrin mRNA
leader sequence of pVL941 is also utilized. The
vector contains two promoters, the polyhedrin promoter

15   of _Autographa californica_ multiple nuclear
polyhedrosis virus (AcMNPV) and the p10 promoter, that
are both activated very late in viral infection. The
gene of interest is cloned downstream of the
polyhedrin promoter which then controls synthesis of

20   the recombinant protein. The vector also contains the
lacZ gene positioned downstream of the p10 promoter
which directs the synthesis of ß-galactosidase. The
two genes, the foreign gene and the lacZ gene, and
their promoters, polyhedrin and p10, recombine with

25   wild-type virus to yield recombinant virus. The
recombinant virus is then plaqued; recombinant virus
generates plaques that are blue and lack occlusion
bodies.

The procedures for transfection of host cells,

30   the subsequent screening and production of recombinant
virus, and ultimately the production of recombinant
protein was performed as described in the MaxBac®™
instruction manual provided with the commercial
purchase of the pBlueBac II expression vector

35   (Invitrogen), the disclosure of which is hereby

incorporated by reference.

Spodoptera frugiperda (Sf9) cells were used for transfecting the recombinant DNA expression vectors of this invention. After 6 days post-viral infection of the cultured cells, the culture plates were examined under a dissecting microscope to select plaques that are occlusion body negative. Wild-type plaques were distinguished by the presence of occlusion bodies. Following selection of occlusion body negative recombinant plaques, the recombinant virus was purified away from contaminating wild-type virus in order to obtain a pure recombinant virus containing either penton base or fiber containing recombinants. Following purification, the selected plaques are analyzed by PCR as described above to confirm the presence of the recombined Ad2 gene and selection of the desired recombinant virus. Once the penton base and fiber recombinant virus were identified, the viruses were propagated to prepare large scale high titer virus stocks as described in the MaxBac®™ manual provided by Invitrogen and with procedures well known to one of ordinary skill in the art. The virus titer was then determined prior to preparation of recombinant penton base and fiber.

Trichoplusia ni (Bti-Tn 5B1-4) cells (hereinafter referred to as Tn 5 cells) were used to express large amounts of recombinant protein since they grow as adherent cell lines and produce recombinant proteins in amounts that surpasses that produced in Sf9 cells. Specifically, two studies identified that Trichoplusia ni attachment-dependent cell line, Tn 5, optimally produced 7 and 26-fold more ß-galactosidase and secretory alkaline phosphatase per cell, respectively, than Sf9 cells. Thus, the Tn 5 cells allowed for the larger-scale protein production

of the recombinant proteins of this invention.

The Tn 5 cells were purchased from Invitrogen and maintained in tissue culture flasks in EX-CELL 400 insect cell medium (JRH Biosciences, Lenexa, KS) supplemented with 2% fetal bovine serum (Irvine Scientific, Santa Ana, CA). The cultures were also adapted for growth in DEAE-based Dormacell 1.2 microcarrier-coated roller bottles (JRH Biosciences) for larger-scale recombinant protein production. For the latter, the microcarriers were added to PBS at 25 mg/ml and autoclaved. Fifty ml of the microcarrier suspension was added to 850 cm^2 polystyrene roller bottles and allowed to attach by slowly rotating the roller bottle at 0.1 rpm. After a confluent layer of microcarriers had attached to the roller bottle surface and just prior to inoculation with the cells, the roller bottle was washed 3 times with 50 ml of sterile saline solution to remove unbound microcarriers. The saline solution was then removed and a suspension of Tn 5 cells was added and allowed to attach and grow at 4 rpm. The volume of cells used for the inoculation was always kept below 100 ml to allow the cells to quickly attach to the microcarriers and to prevent cell clumping. The volume was then adjusted with fresh medium after the cells had attached which was approximately 2-3 hours later.

For expression of the recombinant proteins of this invention, Tn 5 cells were directly plated at densities of 0.75 X 10^4 to 5 X 10^5 cells/cm^2 in 24 well plates (Costar) or on wells pre-coated with Dormacell 1.2 microcarriers. At 0, 1, 2 or 3 days post-plating, the cells were separately infected with the recombinant penton base and fiber pBlueBac II vectors prepared above at a MOI of 5-30 pfu/cell. The cell

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concentration used to calculate MOI after 1, 2 and 3
days of cell growth was estimated by using the cell
doubling time of 24 hours measured for Tn 5 cells in
tissue culture flasks. In the other culturing

5   conditions, Tn 5 cells were also infected with the
recombinant vectors of this invention using an MOI of
5-30 pfu/cell.

    To optimize the level of protein production, an
initial time course was performed. T-25 flasks were

10   seeded with Tn 5 cells at a density of 2 X 10^6
cells/culture. The flasks were then infected with the
high titer viral stock for both recombinant viruses at
a MOI of 5. Aliquots of cells were removed over a
period of 5 days and analyzed for the presence of

15   either penton base or fiber. When the time point at
which maximum protein expression was obtained, larger
scale protein expression was then performed in T-150
flasks seeded with cells at a density of 2 X 10^6
cells/ml and infected with the viral stock to a MOI of

20   5. The infected cells were then harvested by
centrifugation at 4 days post-infection. The
supernatant was transferred to a fresh tube and stored
at 4°C. The pellet was stored at -20°C.

    To purify recombinant penton base and fiber

25   proteins from the pelleted cells, the cells were
separately resuspended in 20 ml of 40 mM Tris-Hcl at
pH 7.65 containing the protease inhibitors (aprotinin
at 1 µg/ml, PMSF at 2 mM and leupeptin at 2 µg/ml).
The suspension was then vortexed and placed on ice for

30   10 minutes to form a cell lysate. The cell lysate was
then centrifuged at 15,000 X g to pellet cell debris
and form a recombinant protein-containing supernatant.
Fiber protein was purified from the resultant
supernatant by DEAE Sepharose (Pharmacia, Piscataway,

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NJ). The running buffer for the column chromatography
was 40 mM Tris-Hcl buffer at pH 7.65. The
supernatants were applied to the column and the
recombinant fiber protein was then eluted from the
5   column with a linear gradient of 10 mM to 500 mM NaCl.
Recombinant fiber protein eluted at 70 mM NaCl. The
peak fractions were collected and pooled. The
recombinant fiber protein was concentrated by Amicon
filters.

10   The concentrated recombinant fiber protein was
then further purified by chromatography over a
Superose 6B column by FPLC (Pharmacia) according to
manufacturer's instructions. Approximately 1-5
milligrams (mg) of recombinant fiber of greater than
15   90% purity was obtained following this purification
procedure from 3 T-150 flasks. A single subunit of
the purified individual protein was 62 kilodaltons
(kD) (582 amino acids) under reducing SDS-PAGE. The
fiber protein exists as a trimer of these three
20   identical polypeptides with a resulting molecular
weight of 180 kD.

        Recombinant penton base was purified as described
above for the fiber protein with the only exception
that the penton base eluted from the DEAE Sepharose
25   column at 170 mM NaCl following 2 washes with 80 mM
NaCl. A single subunit of the purified recombinant
penton base was 82 kD under reducing SDS-PAGE.
Approximately 50 mg of recombinant penton base having
a purity of greater than 90% was purified using the
30   above procedure from 3 T-150 flasks. Penton base
exists as a pentamer consisting of 5 identical single
subunits that are noncovalently associated to form a
ring-shaped complex of approximately 90 Å in diameter
with a central cavity of approximately 10-20 Å.

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Example 2

Purification of Penton Base and Fiber Proteins from Adenovirus Type 2

A.   Production of Viral Antigens

For purification of penton base and fiber proteins adenovirus type 2, a representative of Rosen's subgroup III is propagated in KB cell suspension culture. KB cells are maintained at 3 to 5 x 10^5 cells in Eagle's basal medium supplemented with 5% horse serum and infected with a MOI of 50 particles per cell. Cells are harvested 32 hours post-infection and washed three times in phosphate-buffered saline. The resultant cell pellet is resuspended in ten volumes hypotonic 0.01 M Tris-HCl buffer at pH 8.1, 1 mM EDTA and subjected to five cycles of quick freezing and thawing. The cell lysate is then mixed in a Waring-blendor with an equal volume of fluorocarbon Freon 113. The aqueous phase obtained by low-speed centrifugation containing the viral material is centrifuged for 1 hour at 20,000 rev/min on a caesium chloride cushion (density, 1.43 g/ml) in a swinging bucket rotor (Beckman SW 27). Mature virions appear as a visible opalescent band at the top of the CsCl cushion. The band containing the virions is collected and the virus particles are further purified by density-gradient centrifugation in caesium chloride. The supernatant above the virion band in the two successive cycles of centrifugation, is the source of adenovirus-soluble antigens, namely penton base and fiber proteins.

B.   Precipitation by Ammonium Sulfate

A saturated solution of ammonium sulfate at 4°C is added to the supernatant to a final degree of 55% saturation and the precipitate is allowed to form

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for 15 hours at 4°C and pH 6.5. The precipitate
formed is centrifuged at 5000 x g for 20 minutes at
4°C and the pellet is dissolved in the minimum volume
of 0.05 M sodium phosphate buffer pH 6.8 and dialyzed
5   for 72 hours in the cold against 10 volumes of the
same buffer with at least five changes. After
dialysis, the viral antigen solution is cleared of
possible incomplete low-density virus particles
present in the centrifugation supernatant by
10 centrifugation at 110,000 x g for 2 hours and the
final supernatant or crude antigen preparation stored
at -20°C until further fractionation.

      C.   Chromatography on Sephadex Ion Exchanger
            As a second step, the crude antigen
15 preparation dissolved in 0.05 M sodium phosphate
buffer pH 6.8 is fractionated by chromatography on
DEAE-Sephadex A-50 (Pharmacia Fine Chemicals). The
gel is equilibrated with 0.05 M sodium phosphate
buffer pH 6.8 and poured in a jacketed column (25 x
20 2.5 cm) refrigerated at 4°C. The flow rate is
maintained at 12 ml/hour by a peristaltic pump and the
effluent collected into 3 ml fractions. Absorbance is
measured at 278 nm and the gradient slope is
controlled by measurement of resistivity in each
25 fraction. A volume of crude preparation corresponding
to 250 to 300 mg protein is loaded on top of the
column and the gel is rinsed with 100 ml of the
equilibrating buffer. This is then applied a linear
sodium chloride gradient in the 0.05 M sodium
30 phosphate buffer at pH 6.8, ranging from 0.0 to 0.5 M
(500-ml total volume).

      D.   Chromatography on Hydroxyapatite Column
            Hydroxylapatite is prepared as described by
Boulanger et al., Eur. J. Biochem. 39:37-42 (1973).
35 The hydroxylapatite gel is equilibrated by mild

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stirring in 0.01 M potassium phosphate buffer at pH
6.8 and gently poured into a column (20 x 1 cm)
refrigerated at 4°C. Adenovirus-soluble antigen
fractions are dialyzed against the equilibrating
5   buffer and a volume corresponding to 25-30 mg protein
is loaded on top of the hydroxyapatite column. The
column is rinsed with 30 ml equilibrating buffer, then
a 0.01 to 0.30 M (for penton and hexon) or 0.01 to
0.50 M (for fiber) linear gradient of potassium
10   phosphate at pH 6.8 is applied in a total volume of
120 ml. The flow rate is 15 ml/hour and the effluent
collected into 3-ml fractions. Protein concentrations
are determined by the Lowry procedure with bovine
serum albumin as standard.

15   E.   Polyacrylamide Gel Electrophoresis
                  Native samples are electrophoresed at 5 mA
per tube (in disc gel) or 2.5 mA per slot (in slab
gel) in 6% polyacrylamide gel
(acrylamide:bisacrylamide ratio, 30:0.8) buffered with
20   0.375 M Tris-HCl at pH 8.9 and overlayered with a
spacer gel made of 3% polyacrylamide
(acrylamide:bisacrylamide ratio, 10:2.5) in 0.125 M
Tris-HCl buffer at pH 6.8. The electrode buffer is
0.05 M Tris-0.384 M glycine at pH 8.3. Polypeptide
25   analysis is carried out in sodium dodecyl sulfate
(SDS)-containing polyacrylamide gel. Samples are
denatured by heating for 2 minutes at 100°C in an
equal volume of 4% SDS, 10% 2-mercaptoethanol, 6 M
urea and analyzed on 15.5% polyacrylamide slab gel
30   (ratio of acrylamide:bisacrylamide, 50:0.235) overlaid
by a 3% spacer gel (ratio of acrylamide:bisacrylamide,
50:1.33) in the discontinuous SDS-buffer system of
Laemmli (1970). Purified preparations of adenovirus
type 2-derived penton base and fiber proteins thus
35   result for use in this invention.

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Example 3
Formation of Penton Complex with
Purified or Recombinant Fiber and Penton Base

For use in some aspects of this invention, a
recombinant penton complex consisting of recombinant
penton base and fiber was prepared. The purified
recombinant penton base and fiber proteins prepared in
Example 1 were combined in a 1:1 molar ratio in PBS at
4°C and maintained overnight. The resultant
recombinant penton complex consisting of 5 subunits of
penton base and 3 fiber subunits per penton base were
subsequently purified away from noncomplexed
recombinant penton base or fiber proteins over linear
sucrose gradients. Penton complex is also prepared by
combining Ad2-purified penton base and fiber proteins
prepared in Example 2. While the use of the
recombinant proteins are preferred in this invention
as they can be prepared in large quantities, the
Ad2-purified proteins of this invention can be used in
gene delivery systems instead of the recombinant
proteins with equivalent success. Thus, although not
specified in all of the following Examples, either the
recombinant or the Ad2-purified proteins of this
invention can be used to promote successful gene
delivery of exogenous genes.

The confirmation of the formation of a complex
between recombinant penton base and fiber was
performed using by immunoprecipitation using a
monoclonal antibody directed against the fiber
protein. The procedure used was essentially as
follows: First, purified adenovirus penton base was
metabolically labeled with ^{35}S-methionine and purified
on DEAE-Sepharose as described above. The labeled
penton base was then added to unlabeled fiber to form

a labeled complex that was immunoprecipitated by the
anti-fiber monoclonal antibody Fb10. The complex was
analyzed on 10% SDS gel and autoradiographed.

5   The results of this assay demonstrated that the
anti-fiber antibody successfully immunoreacted and
immunoprecipitated a radioactively labelled
(^{35}S-labeled) penton base bound to the fiber as
compared to the following control where no significant
labeled immunoprecipitable band was detected:

10   ^{35}S-labeled penton base maintained with a control
protein, and then immunoprecipitated by anti-fiber
monoclonal antibody Fb10; ^{35}S-labeled penton base
maintained with fiber, followed by immunoprecipitation
with a control antibody; and ^{35}S-labeled penton base

15   maintained with buffer only and immunoprecipitated
with Fb10.

Example 4

DNA Delivery Into Mammalian Cells

DNA transfer has been accomplished by means of

20   receptor-mediated endocytosis pathway as described by
Wu et al., J. Biol. Chem. 262:4429-4432 (1987) and
Wagner et al., Proc. Natl. Acad. Sci., USA
87:3410-3414 (1990). The use of this
cellular-mediated gene transfer system has certain

25   advantages in that it is not toxic to the recipient
eucaryotic cell membrane, DNA can be transferred
repetitively and the DNA can be targeted to specific
cells by the distribution of cell-specific receptors.
For these type of transfer system, bifunctional

30   molecular conjugate gene transfer vehicles that are
synthetically derived are used. One such bifunctional
conjugate is transferrin and poly-L-lysine where the
transferrin ligand binds to transferrin receptors on
the surface of recipient cells and is then

internalized resulting in the cotransportation of the
poly-L-lysine that is a DNA-binding moiety.

This system requires the presence of specifically
expressed cell surface receptors (e.g., transferrin
receptors) in sufficient quantity to provide for
adequate delivery of the DNA-binding moiety. However,
despite the presence of an adequate number of cell
surface receptors, the transfer of exogenous genes has
been limited by the lysosomal targeting of the
endosome-internalized ligand-DNA complexes as
described by Zenke et al., _Proc. Natl. Acad. Sci., USA_
_87_:3655-3659 (1990) and Cotten et al., _Proc. Natl._
Acad. Sci., USA 87:4033-4037 (1990). Adenovirus has
been shown to enter cells in a similar manner as that
of ligand-DNA complexes but they have the capacity to
disrupt the endosome thereby escaping lysosomal
destruction in the cytoplasm. See, Seth et al., _J._
Virol. 51:650-655 (1984) and Seth et al., _Mol. Cell._
Biol. 4:1528-1533 (1984). More recently,
replication-deficient adenovirus has been shown to
augment the gene transfer of transferrin-polylysine
conjugates mediated by the adenovirus-disruption of
the endosome as described by Curiel et al., _Proc._
Natl. Acad. Sci., USA 88:8850-8854 (1991).

Earlier studies utilized recombinant adenoviruses
to facilitate the transfer of genes but the system
only allowed short heterologous DNA segments of less
than 6 kb to be incorporated into the adenovirus
genome as described by Berkner, _Biotechniques_
_6_:616-629 (1988). The system improved by Curiel et
al. _supra_ provided for the transfer of DNA segments of
up to 15 kb without functional constraints. However,
the use of replication-deficient virus to mediate
transfer of foreign genes is still not optimal as
co-infection of epithelial cells with wild-type

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strains of virus may result in transactivation of the
replication-deficient virus which thus presents a
significant safety concern for *in vivo* applications.
Furthermore, whether recombinant adenoviruses are

5   capable of integrating their genome into the host cell
DNA allowing for long-term stable expression of the
foreign gene has not yet been determined.

       The use of recombinant penton base and fiber
proteins of this invention to mediate the transfer of

10   foreign genes into recipient cells both *in vitro* and
in vivo overcomes the limitations of the
above-described gene transfer systems. This invention
utilizes a recombinant coat protein subunit of
adenovirus, the penton, which duplicates the cell

15   receptor binding and DNA delivery properties of intact
adenovirus virions and thus represents an improved
method for gene therapy as well as for antisense-based
antiviral therapy. The ability of penton base and
fiber to mediate the transfer of exogenous or

20   non-native genes into cells was first evaluated in an
in vitro tissue culture system. The DNA for transfer
in the *in vitro* assay was a DNA plasmid containing the
luciferase gene, pRSVLuc. The ligand-DNA complex,
transferrin-poly-L-lysine, was also used in each

25   transfer experiment as it allowed for the detection of
gene transfer from the cell endosome to the cytoplasm.
Luciferase activity was then assayed at 48 hours post
transfection.

       For this assay, monolayers of Hela cells were

30   plated onto type IV collagen immobilized on tissue
culture dishes and maintained in Dulbecco's modified
Eagle's medium (DMEM) containing 5% heat-inactivated
fetal bovine serum, penicillin at 100 international
units/ml, streptomycin at 100 μg/ml and 2 mM

35   glutamine.

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The DNA plasmid, pRSVLuc, containing the *Photinus pyralis* luciferase gene under the control of the Rous sarcoma virus long terminal repeat enhancer/promoter was used as a reporter gene. The pRSVLuc was prepared

5   as described by DeWet et al., Mol. Cell. Biol. 7: 725-737 (1987), the disclosure of which is hereby incorporated by reference. Other reporter genes such as those expressing ß-galactosidase, alkaline phosphatase and chloramphenicol acetyltransferase are

10   contemplated for use *in vitro* gene transfer experiments as described herein and are well known to one of ordinary skill in the art.

Human transferrin-poly-L-lysine conjugates with an average chain length of 190 lysines were

15   synthesized as described by Wagner et al., Proc. Natl. Acad. Sci., USA 87:3410-3414 (1990) and Wagner et al., Bioconjugate Chem. 2:226-231 (1991), the disclosures of which are hereby incorporated by reference. Briefly, a solution of 102 mg (1,28 μM) of human

20   transferrin in 3 ml of a 30 mM sodium acetate buffer at pH 5 was subjected to gel filtration on a Sephadex G-25 column (Pharmacia). Following isolation of oxidized transferrin, the solution was added to a solution of 0.50 μM of poly-L-lysine. The resultant

25   formed conjugates were then purified on a Mono HR 10/10 S column (Pharmacia) with a high salt gradient elution. To prepare transferrin-poly-L-lysine-pRSVLuc complexes (also referred to as conjugate-DNA complexes), 6 μg of pRSVLuc DNA in 350 μl of HBS (150

30   mM NaCl and 20 mM Hepes at pH 7.3) were admixed with 12 μg of the transferrin-poly-L-lysine conjugate diluted in 150 μl of HBS. Complexes were allowed to form for 30 minutes at room temperature before admixing with cells.

35   For the gene transfer experiments, the cells

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prepared as described above were grown in 10
centimeter tissue culture plates until approximately
50% confluent (5 X 10^6 cells). Medium was removed and
1 ml of DMEM or Eagle's minimal essential medium
5   containing 2% fetal bovine serum was then added.
Conjugate-DNA complexes prepared above were then
admixed with the cells followed by admixture of 50 µg
of either recombinant penton base, recombinant fiber
or both recombinant proteins together. For
10   experimental controls, in one case only the
transferrin-poly-L-lysine without the pRSVLuc DNA or
the recombinant proteins alone was added to the cells
while in the other control, the conjugate-DNA complex
was added in the absence of the recombinant proteins.
15   After the admixtures, the plates were returned to
the incubator (5% CO_2 at 37°C) after which 3 ml of
complete medium was added. After an additional 48
hours, the cells were harvested by lysis by freezing
and thawing cycles for analysis of luciferase gene
20   expression on a luminescence photometer according to
manufacturer's instructions (Analytical Luminescence
Laboratory, San Diego, CA).
          As shown in Figure 3, DNA delivery into cells via
the adenovirus penton base is illustrated. Luciferase
25   activity was measured and plotted against sample
number, as shown. The sample numbers are listed on
the X-axis while luciferase activity (in light units)
is plotted on the Y-axis. The samples identified by
numbers 1-5 contained the following:
30   (1) transferrin-poly-L-lysine (shown as
transferrin/PL), but no pRSVLuc; (2) pRSVLuc +
transferrin/PL; (3) pRSVLuc + transferrin/PL + penton
base + fiber; (4) pRSVLuc + transferrin/PL + penton
base; (5) pRSVLuc + transferrin/PL + fiber.

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A DNA plasmid containing the luciferase gene,
pRSVLuc was added to monolayers of HeLa cells which
had been previously plated onto type IV collagen. DNA
delivery was carried out in the presence of
5   transferrin/poly L-lysine to allow detection of gene
transfer from the cell endosome to the cytoplasm.
Luciferase activity was assayed at 48 hours post
transfection. (Refer to Example 3 for details of the
gene transfer experiment.)
10   The samples containing recombinant penton base
both demonstrated successful DNA delivery into the
cells as measured by luciferase activity. Sample 4,
which contained recombinant adenovirus fiber as well
as penton base, displayed a somewhat greater
15   introduction of DNA into the cells. The results of
the assay using sample 5 demonstrates that adenovirus
fiber alone, unaccompanied by penton base, does not
appear to promote DNA delivery into cells. With HeLa
cells with pRSVLuc plus transferrin-poly-L-lysine, in
20   the absence of either penton base or fiber, no
luciferase activity was detected which indicates
internalization of the transferrin complex alone via
transferrin receptors is not sufficient to allow gene
delivery. Thus, penton base was an efficient means of
25   mediating gene transfer in cells which do not
efficiently allow gene delivery into the cytoplasm
following internalization of the transferrin by the
coordinate ligand.

Example 5
30   Construction of Penton Conjugates for Gene Delivery

Both *in vitro* and *in vivo* studies on the
replication-deficient adenovirus-mediated transfer of
foreign genes encoding either alpha 1-antitrypsin or
cystic fibrosis transmembrane regulator protein have
35   been performed on rat lung epithelial tissues for

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local as well as systemic use as described in NTIS
Publication PB92-139260 (Pat-Appl-7-769-623), the
disclosure of which is hereby incorporated by
reference. The use of recombinant penton base or
5   penton complex is contemplated within the context of
the use of replication-deficient adenovirus to
transfer exogenous genes into the lung for replacement
of defective or nonexpressed genes. The advantages of
using the recombinant proteins of this invention as
10   described in Example 4 are applicable in this context
of both in vitro and in vivo utility. For in vitro
gene transfer, administration is accomplished by first
isolating a selected cell population from a patient
such as lung epithelial cells, lymphocytes and the
15   like followed by in vitro gene transfer of the
therapeutic compositions of this invention and the
replacement of the cells into the patient. In vivo
therapy is preferred through the administration of the
therapeutic compositions of this invention by aerosol
20   means. Also contemplated for in vivo applications are
methods of administering therapeutic compositions of
this invention by subcutaneous, intravenous,
intraperitoneal, intramuscular, ocular means and the
like.

25   One possible mode of penton-mediated gene
delivery may be based on coating the penton base or
penton complex with poly-L-lysine, which allows
formation of a complex with DNA as well as binding to
cell receptors. The low pH in the endosome causes
30   penton base-mediated disruption of the endosomal
membrane and release of the DNA plasmid into the
cytoplasm.

In addition to the mode of application of penton
base or penton complex in facilitating the transfer of
35   exogenous genes into the lungs as described above,

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other means are contemplated to achieve the same
results in both *in vivo* and *in vitro* applications.
One gene delivery system utilizing the recombinant
adenovirus penton complex results in the transfer of
5   genes through the use of an avidin-biotin complexation
reaction and an antibody bridge to either recombinant
fiber, penton base or penton complex.

For this system, the required reagents include
the following: either purified or
10   recombinant-produced fiber and penton base proteins
used separately or non-covalently bound into penton
complex; a nonfunctional blocking antibody, preferably
a monoclonal antibody, that immunoreacts with either
fiber or penton base proteins so that it does not
15   inhibit the protein's functional activity; a
biotinylated CIAP (calf intestinal alkaline
phosphatase expression vector) into which the
exogenous gene or therapeutic gene or foreign DNA sequence
of interest has been inserted; and a chimeric protein
20   consisting of Protein A linked to streptavidin
(referred to as SA-PA).

Since the CIAP vector DNA encodes alkaline
phosphatase, the delivery of this gene into the cell
allows can be measured by quantitative colorimetric
25   assays much in the same fashion as ß-galactosidase and
the like. Biotinylation of one nucleotide on the
plasmid allows its binding to the SA-PA chimeric
protein in a defined way. Each SA-PA molecule can
bind 4 biotin moieties linked to DNA. An expression
30   vector capable of producing SA-PA in bacteria has been
previously described by Sano et al., Science
258:120-122 (1992) and Sano et al., Bio/Technology
9:1378 (1991). Antibodies used in this invention
include monoclonal and monospecific polyclonal
35   antibodies that immunoreact with domains on the fiber

or penton base proteins such that the protein's
biologic functions are not inhibited. For example,
see, Boudin et al., *Virol. 92*: 125-138 (1979).
Alternative monoclonals to nonfunctional domains of
5   α-v integrins such as LM142 described by Cheresh et
al., *J. Biol. Chem. 262*:1434-1437 (1987) are also
contemplated for used in this invention.

In this gene delivery system, the biotinylated
DNA plasmid containing the therapeutic DNA sequence of
10   interest binds to the streptavidin-Protein A chimeric
protein. The DNA-bound SA-PA complex then binds via
the PA moiety to an antibody that immunoreacts with
either the fiber or penton base (also referred to as
ligands). The ligands are either used separately in
15   the noncomplexed form or used in the complexed form
where fiber protein is noncovalently bound to penton
base. If the fiber protein is used in the
noncomplexed form to bind to an anti-fiber antibody,
following the formation of the antibody and fiber
20   bridge, the products are then admixed with penton base
to form penton complex. Thus, a penton complex (also
referred to as penton) is ultimately formed
irrespective of whether the proteins of this invention
are used in the noncomplexed form initially.

25   The formed PA-immobilized antibody-ligand complex
then binds, via the functional receptor-binding site
on the ligand, with the ligand-specific receptors
expressed on the recipient cell surface. The
resultant ligand-occupied receptors then provide for
30   the delivery of the exogenous or foreign genes/DNA
sequence(s) into the cell via the specific functions
of the adenovirus-derived proteins of this invention.

In this gene delivery system described herein,
the fiber protein binds to fiber-specific receptors
35   the binding of which provides for nuclear localization

signal targeting following receptor internalization.
Following the fiber-mediated internalization process,
the penton base protein, that is either admixed
separately with the fiber-antibody bridge-PA-SA-DNA
complex or is already noncovalently bound to fiber
prior to the formation of the antibody bridge complex,
provides for the enhanced uptake of DNA into the
cytoplasm via the release from the endosome.

In addition to the methods of gene delivery
described above, also contemplated is the use of
penton base without fiber protein in the formation of
the antibody-PA-SA-biotinylated DNA plasmid complex.
The amino acid residue sequence present in the penton
base, arginine-glycine-aspartic acid (RGD) is used to
target the penton base to RGD-specific receptors
expressed on cell surfaces. The RGD peptide sequence
present in penton base has been shown to be the ligand
binding site recognized by integrin receptors such as
the vitronectin and fibronectin integrin receptors.
When cells such as lymphocytes that lack RGD-specific
receptors are cultured on synthetic RGD-coated plates
in the presence of high concentrations of manganese,
the expression of fibronectin receptor results thereby
resulting in the binding of the RGD-containing penton
base to the RGD-specific fibronectin receptor. The
resultant receptor-penton base ligand interaction
causes a consequent enhancement of internalization of
occupied-cell surface receptors and increased
permeability of the endosome.

Many advantages result from the use of the
above-described gene/DNA delivery system. Multiple
copies of the exogenous DNA in the biotinylated DNA
plasmid are targeted to recipient cells. The
recombinant fiber or penton base protein provides for
targeting of the foreign gene to epithelial,

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endothelial, platelets and lymphoid cells expressing
fiber-specific receptors. The recombinant penton base
enhances the uptake of foreign DNA and the release
from the endosome evading destruction by lysosomal
5   enzymes. If the gene transfers are performed on
cultured cells in vitro for the ultimate replacement
into a patient requiring gene therapy, the cells can
be plated on a synthetic RGD-coated surface that
results in the upregulation of the fibronectin
10 receptors (alpha$_5$ beta$_1$) thereby augmenting
adenovirus-protein mediated uptake. As mentioned
above, targeting to nonepithelial cells, such as a
patient's lymphocytes, is accomplished by plating
those cells on RGD in the presence of high
15 concentrations of manganese which upregulates
fibronectin receptors that will bind penton base RGD.
      Also contemplated for use in this invention are
the alternative means of gene delivery where genes,
DNA oligonucleotide sequences such as those encoding
20 ribozymes, or antisense-based plasmids are conjugated
directly to a region of the penton (penton base plus
fiber) such that the gene delivery functions of the
proteins are not inhibited. The plasmid-based vectors
can either be under the control of strong constitutive
25 promoters or regulatable promoters along with enhancer
elements to obtain higher levels of gene expression
than that obtained by incorporating the therapeutic
gene into the intact or replication-deficient genome.
Constitutive promoters include human cytomegalovirus
30 which contains an enhancer sequence while regulatable
promoters include those that are regulated by
chemicals or temperature. Chemically regulatable
promoters for eucaryotic expression systems include
those based on induction by γ-interferon, heat-shock,

heavy metal ions, and steroids such as glucocorticoids
as described by Kaufman et al., In "Current Protocols
in Molecular Biology", Ausubel et al., eds., Unit
16.12.5, New York (1990). For a review of strategies
5   for expressing high-level protein expression in
mammalian cells see Kaufman, _Technique 2_: 221-236.
(1990), the disclosure of which is hereby incorporated
by reference.

Alternative gene delivery systems are also
10   contemplated where the penton is incorporated into
liposomes which contain either the therapeutic foreign
gene(s) or antisense oligonucleotides. An extension
of this invention is that the adenovirus penton is
used to target and deliver genes into nonepithelial
15   cells by incorporating the attachment sequence
(receptor-binding site) present in ligands specific
for other receptors onto the fiber protein by
recombinant DNA techniques for the production of a
chimeric protein molecule as described herein. The
20   chimeric molecule is referred to as a penton
complex-ligand conjugate or a penton base-ligand
conjugate depending on whether the proteins are used
in the complexed or noncomplexed form. The chimeric
molecule is then complexed via the fiber protein to
25   the anti-fiber antibody-(PA-SA)-biotinylated DNA
containing the exogenous DNA prepared as described
above. The presence of preselected attachment
sequences in the resultant complex provides for
targeting to cells expressing specific receptors.
30   Examples of receptors and their respective ligands or
attachment sequences for use in gene delivery include
the following: CR2 receptor binding to the amino acid
residue attachment sequences EDPGFFNVE (SEQ ID NO 6)
and EDPGKQLYNVE (SEQ ID NO 7); CD4 receptor
35   recognizing the V3 loop of HIV gp120; transferrin

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receptor and its ligand transferrin; low density
lipoprotein receptor (LDL) and its ligand; and
asialoglycoproteins that recognize deglycosylated
protein ligands.

Instead of incorporating receptor attachment
sequences, antibodies specific to preselected cell
surface receptors can also be used in the gene
delivery system described above. Antibodies defined
for use in this manner include immunoglobulin
molecules and immunologically active portions of
immunoglobulin molecules such as those containing an
antibody combining site or paratope. Exemplary
antibodies are intact immunoglobulin molecules,
substantially intact immunoglobulin molecules and
portions of an immunoglobulin molecule, including
those portions known in the art as Fab, Fab', F(ab')2
and F(v). Antibodies can be produced by conventional
techniques for preparing polyclonal and monoclonal
antibodies and also by recombinant DNA techniques
familiar to one of ordinary skill in the art and as
described in US Patent 4,816,567 and International
Application WO 90/14424. Antibodies specific for cell
surface receptors for use as described herein include
antibodies to integrins, MHC class I and class II,
asialoglycoprotein receptor, transferrin receptor, LDL
receptor and the like.

The gene delivery systems utilizing the
adenovirus-derived penton base and fiber proteins of
this invention are exemplary means for therapeutic
intervention of common hereditary disorders involving
respiratory epithelium including emphysema and cystic
fibrosis. In addition, this invention is useful for
the delivery of antisense compounds to epithelial
cells. Since a large number of human pathogens
including rhinoviruses, rotoviruses, herpesviruses,

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and papilloma virus invade the host via the epithelial
cell route as a primary site of infection, the ability
to target antiviral agents to epithelial cells is of
therapeutic importance. The use of adenovirus-derived
5   penton gene delivery systems is also valuable for the
delivery of immunotoxin for the treatment of various
cancers, including melanomas or osteosarcomas.

Example 6

Targeting Specific Cells

10   As discussed in the aforementioned Examples, the
present invention contemplates that the therapeutic
compounds and compositions of the present invention
may be directed to specific receptors or cells, for
the ultimate purpose of delivering those compounds and
15   compositions to specific cells or cell types. The
adenovirus-derived proteins and polypeptides of the
present invention are particularly useful in this
regard.

Adenovirus attachment and uptake into cells are
20   separate but cooperative events that result from the
interaction of distinct viral coat proteins with a
receptor for attachment and α_v integrin receptors for
internalization. In the present invention, adenovirus
attachment to the cell surface via the fiber coat
25   proteins has now been discovered to be dissociable and
distinct from the subsequent step of internalization,
an event that depends on α_v integrin binding to the
RGD-containing penton base viral coat protein. These
findings represent the first evidence for the
30   existence of a virus internalization receptor which is
distinct from, and functions independently of, a virus
attachment receptor.

Integrins which recognize the RGD motif include
$\alpha_5\beta_1$, $\alpha_{IIb}\beta_3$, and most, if not all, α_v-containing

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integrins (i.e., vitronectin receptors). Integrins
have also been implicated in the attachment of certain
viruses and bacteria to host cells. For example, the
collagen/laminin-binding integrin, $\alpha_2\beta_1$, has been
5   identified as a receptor for echovirus 1 attachment
(Bergelson et al, _Science 255_: 1718-1720, 1992), while
Yersinia (Isberg, _Science 252_: 934-938, 1991) and
Bordatella pertussis (Relman et al, _Cell 61_:
1375-1382, 1990) have been shown to bind to and
10   internalize into host cells via $ß_1$ and $ß_2$ integrins,
respectively. The receptor for internalization of
adenovirus has not been identified.

Vitronectin receptor is an integrin that mediates
cell adhesion and other cellular receptor functions.
15   Vitronectin receptor is a heterodimeric receptor
defined by the presence of an α_v subunit and a second,
β subunit, typically β_3 or β_5. Ligands for the
vitronectin receptor are arg-gly-asp (RGD)-containing
polypeptides or proteins, such as the adenovirus
20   penton base.

In general, integrins may be classified in a
relatively simple fashion based on their binding
characteristics, as shown in Table 1.

25   Table 1
Subunit   Ligands
_____

Group 1[1]
30
$\alpha_1/ß_1$ Laminin/collagen

$\alpha_2/ß_1$ Collagen/laminin

35      $\alpha_3/ß_1$ Laminin/collagen/fibronectin

$\alpha_6/ß_1$ Laminin

$\alpha_6/ß_4$ Laminin

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(Table 1, cont'd)

Group 2[2]

α_4/β_1	Fibronectin (CSII site)
α_5/β_1	Fibronectin (RGD site)
α_v/β_1	Fibronectin
α_v/β_3	Fibronectin, vitronectin, fibrinogen, thrombospondin, von Willebrand's factor
α_v/β_5	Vitronectin
α_v/β_6	Fibronectin
α_v/β_8	Unknown

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[1] Integrins that bind primarily to basement membrane proteins

[2] Integrins that bind primarily to matrix proteins of inflammation, wound healing, and development

A. Vitronectin Receptor Ligands as Targeting and Delivery Agents

Thus, as further disclosed herein, the binding interaction between penton base and the vitronectin receptor is RGD-dependent. Thus, an adenovirus penton base protein, and polypeptides derived therefrom which contain at least the RGD domain of the penton base protein, are expected to be useful in directing the therapeutic nucleotide sequences of the present invention to the vitronectin receptor.

Thus, the invention also contemplates isolated penton base protein, penton base fragments, and penton base-derived polypeptides which contain the RGD domain of adenovirus penton base. Particularly preferred are (1) isolated penton base protein described herein

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produced by recombinant DNA methods or produced by
purification from adenovirus stock sources, (2)
polypeptides having the RGD sequence and that
substantially bind vitronectin receptor, and (3)
5   compositions comprising one or more of the above
ligands.

The present invention contemplates the use of any
vitronectin receptor ligand as an active ingredient in
a therapeutic composition according to the present
10   invention, although adenovirus penton-derived proteins
or polypeptides are particularly preferred. A
vitronectin receptor ligand can be any polypeptide,
protein, polypeptide or protein derivative, fragment
or analog thereof which has the ability to
15   substantially bind to vitronectin receptor.

"Substantially bind" in the present context means
a binding affinity sufficient to be useful in the
methods of the present invention. As is known in the
art, useful competition for binding to a receptor
20   depends on both the binding affinity and the
concentration of ligand achievable in the vicinity of
the receptor. Thus, binding affinities lower than
that found on a natural ligand such as isolated
adenovirus penton base are useful, so long as the cell
25   or tissue to be treated can tolerate concentrations of
ligand sufficient to compete with adenovirus binding
to the receptor. Natural binding affinity of isolated
penton base was determined to have a dissociation
constant (Kd) of 55 nanomolar.

30   A preferred adenovirus-derived protein or
polypeptide has a sequence that corresponds to
adenovirus strain 2 or 5 (Ad2 or Ad5). Another
preferred adenovirus-derived protein or polypeptide
for use as a vitronectin receptor ligand according to
35   the present invention includes the sequence

represented by the formula -HAIRGDTFA- (SEQ ID NO 1).

Synthesis of useful polypeptides according to the
present invention can be accomplished by a variety of
well known methods, including the procedures described
hereinabove. Methods for identifying other suitable
vitronectin receptor ligands useful in the present
compositions, such as additional polypeptides based on
the sequence of an adenovirus penton base amino acid
residue sequence, can be identified by the use of
cell binding assays described in the above Examples.

     B.   Penton Base as a Targeting and Delivery
          Agent

          1.   Interaction with Integrins

The penton base coat protein contains five RGD
sequences and was thus postulated to interact with
cell surface integrins. To determine whether the
adenovirus penton base binds to integrins, cell
adhesion experiments were performed on surfaces coated
with various cell matrix proteins or with recombinant
penton base. The cell adhesion assay to extracellular
matrix proteins and to recombinant Ad2 penton base was
performed using three different cell lines. The M21
melanoma cell line was obtained from Dr. Donald Mortan
(University of California at Los Angeles, CA). The
M21-L4 (α_v-expressing) and M21-L12 (α_v-deficient) cell
lines were derived from the M21-L cell line as
described by Cheresh et al., J. Biol. Chem. 262:
17703-17711 (1987) and characterized for their
integrin expression as previously described by
Felding-Habermann et al., J. Clin. Invest. 89: 1-5
(1992). HeLa cells (ATCC Accession No CCL2) and the
A549 lung carcinoma cell line (ATCC Accession No
CCL185) were obtained from the American Type Culture
Collection (ATCC) (Rockville, MD).

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For the assay, the cells were first labeled with
^{3}H-thymidine.   Individual wells of 48 well
non-treated cluster plates at a concentration of 5 X
10^4 cells/well (Costar, Cambridge, MA) were first
5   pre-coated overnight with varying amounts of
extracellular matrix proteins or penton base.
Vitronectin, laminin, fibronectin, and collagen I were
purchased from Sigma (St. Louis, MO). Recombinant
penton base was prepared as described in Example 1.
10   The wells were separately coated with vitronectin (10
μg/ml), collagen I (10 μg/ml), laminin (10 μg/ml), or
penton base (0.5 μg/ml).

The coated wells were then blocked with 5% BSA in
PBS at pH 7.4 for 1 hour. The versene-released
15   labeled HeLa, M21, or A549 cells resuspended in
adhesion buffer (DMEM supplemented with 2 mM $MgCl_2$, 1%
BSA, and 20 mM HEPES) were then added and allowed to
attach at 37°C for 1 hour, the time at which adhesion
reached a maximum in wells containing the most penton
20   base and vitronectin. Unattached cells were removed
by rapid washing with PBS and the amount of
cell-associated radioactivity remaining in each well
was determined by addition of detergent and
scintillation counting the cell lysates. The
25   percentage of attached cells was calculated from the
total cell cpm added to each well.

In the cell attachment assays, HeLa, M21, and
A549 cells efficiently attached to vitronectin,
collagen and laminin. In addition, these cells also
30   attached to and spread on recombinant penton
base-coated wells.

To determine the effect of synthetic peptides on
cell adhesion, the wells were coated with 0.5 μg/ml
penton base or 10 μg/ml of vitronectin, laminin, or

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collagen I and then blocked with BSA. Cells were
pretreated with 150 µg/ml of synthetic peptides (RGD-
containing or non-RGD-containing, e.g., GRGDSP or
GRGESP) in adhesion buffer for 1 hour at 4°C and then
5   allowed to attach to the wells at 37°C for 30-60
minutes. The percentage of attached cells was
determined as above.

Synthetic peptides useful as described above are
commercially available. For example, RGD-containing
10   (e.g., GRGDSP) and non-RGD-containing (e.g., GRGESP)
peptides are available from Telios Pharmaceuticals,
Inc. (San Diego, CA). Alternatively, synthetic
peptides may be prepared via well-known techniques,
such as the classical solid-phase technique described
15   by Merrifield, Adv. Enzymol. 32: 221-296 (1969) as
adapted for use with a model 430 automated peptide
synthesizer (Applied Biosystems, Foster City, CA).
Prepared polypeptide resins are cleaved by hydrogen
fluoride, extracted and analyzed for purity by
20   high-performance liquid chromatography (HPLC) using a
reverse-phase C18 column manufactured by Waters
Associates (Milford, MA).

In these competition assays, cell attachment to
penton base as well as vitronectin was abrogated by
25   incubating cells with the integrin-specific (i.e.,
RGD-containing) synthetic peptide, but not by a
control peptide (i.e., non-RGD-containing). As
expected, the RGD-containing peptide did not affect
adhesion to collagen, since most collagen-mediated
30   adhesion can be potentiated by RGD-independent
integrins (Hynes, Cell 69: 11-25 (1992). Cell
attachment to all three matrix proteins and to penton
base was blocked by the divalent metal ion chelator
EDTA. These results suggested that penton base binds
35   to the cell surface and this event is mediated by an

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RGD-dependent integrin receptor.

      2.   <u>Penton Base Binds to α_v Integrins</u>

     To demonstrate a specific role for α_v integrins in adenovirus infection, a defined pair of M21 melanoma cells were used that either express or lack α_v integrins. M21-L cells specifically lack α_v mRNA and protein as described by Cheresh et al., <u>J. Biol. Chem. 262</u>: 17703-17711 (1987). The cells were prepared as described in Section A immediately above and transfected with a full length α_v cDNA, designated M21-L4 or mock-transfected, designated M21-L12. Thus, M21-L4 cells expressing α_v attach to vitronectin using $\alpha_v\beta_3$ and $\alpha_v\beta_5$ while M21-L12 cells, which fail to express these integrins, do not (Felding-Habermann et al., <u>J. Clin. Invest. 89</u>: 1-5 (1992). Both cell lines express β_1 integrins and thus attach to collagen, laminin, and fibronectin (Felding-Habermann et al., supra.

     In the cell attachment assay performed as described in Section A hereinabove, the adhesive properties of α_v-transfected (M21-L4) and α_v-negative (M21-L12) cells and inhibition by RGD-containing peptides and anti-α_v integrin monoclonal antibodies (also referred to as mAbs) were evaluated. Tritiated-thymidine-labeled M21-L4 and M21-L12 cells were allowed to attach to polystyrene wells precoated with concentrations ranging from 0.01 to 10 μg/ml of vitronectin or penton base at 37°C. Binding of both cell lines to collagen I was included as a control. After 30 minutes, the wells were washed and cell adhesion was determined as the percentage of cells (cpm) attached compared with the total number of cells (cpm) added to each well. Values are the average of duplicate measurements.

     Adhesion of M21-L4 or M21-L12 to polystyrene

wells precoated with varying doses of either
vitronectin or penton base was examined. M21-L4 (α_v+)
but not M21-L12 (α_v-) cells adhered to vitronectin in
a dose-dependent manner while both cell types bound to
collagen I (data not shown). M21-L4 (α_v+) cells, but
not M21-L12 (α_v-) cells, also adhered to penton base
although a small amount M21-L12 binding to the highest
penton base concentrations was observed. This
low-level binding was inhibitable with an RGD-
containing peptide (data not shown) suggesting it was
due to interaction with another RGD-binding receptor,
such as $\alpha_5\beta_1$ expressed on these cells as described by
Felding-Habermann et al., supra.

     To further substantiate the role of α_v integrins
in penton base binding, synthetic peptides or
monoclonal antibodies (MAbs) specific for α_v integrins
were used to examine cell interaction with penton base
or adenovirus infection of cells. M21-L4 (α_v+) cells
were incubated with function-blocking MAbs to the
vitronectin receptors, $\alpha_v\beta_3$ (LM609) and $\alpha_v\beta_5$ (P3G2), a
control, non-function-blocking MAb to the α_v subunit
of α_v integrins (LM142) or with an RGD-containing
peptide and then examined for adhesion to penton
base-coated surfaces. The LM142 monoclonal antibody
(MAb) directed against a nonfunctional epitope of α_v
integrins was produced as previously described
(Cheresh et al., J. Biol. Chem. 262: 1434-1437 (1987).
The MAbs LM609, P3G2, and 9227 directed to $\alpha_v\beta_3$ and
$\alpha_v\beta_5$, and α-proteoglycan core protein respectively,
were produced as previously described. See, Cheresh
et al., J. Biol. Chem. 262: 17703-17711 (1987); Wayner
et al., J. Cell Biol. 113: 919-929 (1991); and Bumol
et al., PNAS USA 79: 1245-1249 (1982).

     For the competition of cell attachment assays,

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^{3}H-thymidine-labeled M21-L4 cells were first incubated
with RGD-containing and control (i.e., non-RGD-
containing) peptides (200 μg/ml) or with the indicated
MAbs (100 μg/ml) for 1 hour at 4°C and the cells were
then added to penton base-coated wells (0.5 μg/ml) for
60 minutes at 37°C. Unattached cells were removed by
washing and cell adhesion was quantitated by
determining the percentage of the total cells (cpm)
added that had attached (cpm). Values were the
average of duplicate measurements.

The results indicated that RGD-containing
peptide, but not the control peptide, inhibited M21-L4
adhesion to penton base by greater than 95% (data not
shown). Cell adhesion to penton base was also
significantly inhibited by a combination of the
functional blocking anti-α, MAbs, P3G2 and LM609 but
not by the non-functional MAb LM142 or by the P3G2 or
LM609 antibodies alone. Similar results were obtained
with HeLa and A549 cells. The MAb P4C10, directed
toward the functional epitope on a ß1 integrin, did
not significantly affect adhesion of any of the cell
lines to penton base. These results demonstrate that
α$_v$ integrins, α$_v$β$_3$ and/or α$_v$β$_5$, are the primary
receptors for the viral penton base coat protein.

        3.   <u>Penton Base Interaction with Integrins</u>
                <u>Induces Membrane Permeabilization</u>
              a.   <u>Cell Lines and Assays</u>

HE-p-2, HeLa and H2921 cell lines were obtained
from ATCC (Rockville, MD). The CS-1 hamster melanoma
cell line (Farishian, et al., <u>Arch. Biochem. Biophys.</u>
<u>198</u>: 449-461 (1979)), a generous gift of Dr. Carolyn
Damsky (UCSF, San Francisco, CA) was propagated in
RPMI supplemented with 10% fetal bovine serum, 2mM L-
glutamine, and 50μg/ml gentamicin. CS-1 cells do not

adhere to vitronectin due to their failure to express
α_v integrins (Thomas, et al., _J. Cell. Sci. 105_: 191-
201 (1993)). These cells express neither $\alpha_v\beta_3$ nor $\alpha_v\beta_5$
heterodimers on their surface yet maintain an

5   intracellular pool of the α_v integrin subunit. To
generate CS-1 sublines expressing either $\alpha_v\beta_3$ or $\alpha_v\beta_5$
integrins on their surface, cDNAs encoding full-length
β_3 (Fitzgerald, et al., _J. Biol. Chem. 262_: 3936-3939
(1987)) or β_5 (Ramaswamy, et al., _EMBO J. 9_: 1561-1568

10   (1990)) subunit proteins were subcloned into the
pcDNA-1/NEO expression vector (Invitrogen, La Jolla,
CA) and introduced into CS-1 cells via lipofectin-
mediated transfection (Gibco-BRL, Gaithersburg, MD).
Stably transformed cells were selected by growth in

15   500μg/ml Geneticin (Sigma, St. Louis, MO) and enriched
from the neomycin-resistant population for the
expression of either $\alpha_v\beta_3$ or $\alpha_v\beta_5$ by selectively
propagating the adherent cell population and
discarding the unattached cells during passage of the

20   cell line.
       Human adenovirus type 2 (Ad2) was propagated in
HeLa or H2981 cells and was purified and stored as
previously described (Everitt, et al., _J. Virol. 21_:
199-214 (1977)). Briefly, Ad2 was banded on cesium

25   chloride gradients and then dialyzed against 10mM
phosphate-buffered saline (PBS), pH 8.0, containing
10% glycerol and 1mM $MgCl_2$. Purified virus was stored
at -70°C and dialyzed into the appropriate buffer just
prior to use. Ad2 was labeled with [^{3}H]-thymidine as

30   previously described (Svensson, et al., _J. Virol. 38_:
70-81 (1981)) or with NHS-SS-biotin (Pierce, *) as
recommended by the manufacturer.
       The LM142 monoclonal antibody (mAb) directed
against a nonfunctional epitope of α_v integrins

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(Cheresh, et al., _J. Biol. Chem. 262_: 1434-1437
(1987)) and the function-blocking mAbs LM609, P3G2,
and P4C10 directed to $\alpha_v\beta_3$, $\alpha_v\beta_5$, and β_1, respectively,
were produced as previously described (Cheresh, et
al., _J. Biol. Chem. 262_: 17703-17711 (1987); Wayner,
et al., _J. Cell. Biol. 113_: 919-929 (1991)).

   Cell adhesion assays were carried out as
described in sections A and B hereinabove. Ad2 virus
infection was quantitated using the fluorescent focus
assay (Thiel, et al., _Proc. Soc. Exp. Biol. Med. 125_:
892-895 (1967), incorporated by reference herein).

   Binding of ³H-thymidine-labeled Ad2 or ³⁵S-penton
base to CS-1:ß3 and CS-1:ß5 cells was carried out as
previously described (Wickham, et al., _Cell 73_: 303-
313 (1993)). Nonspecific binding was determined in
the presence of 50-fold excess unlabeled Ad2 or penton
base.

   Ad2 internalization into CS-1:ß3 and CS-1:ß5
cells was performed with biotinylated Ad2 using a
capture ELISA as previously described by Smythe, et
al. (_Meth. Enzymol. 219_: 223-234 (1992)). Briefly,
60µg of biotinylated Ad2 was added to 1×10^7 CS-1:ß₃
or CS-1:ß₅ cells for 1 hour at 4°C in adhesion buffer.
The unbound virus was removed by washing, and cell
samples of 1×10^6 cells each were warmed to 37°C for
varying lengths of time. Uninternalized virus
particles remaining on the cell surface were then
"quenched" by the addition of 100µg/ml of soluble
avidin (Boehringer-Mannheim, Indianapolis, IN) for 60
minutes at 4°C in HEPES-buffered saline containing
10mM EDTA. Internalized Ad2 was then released by
solubilizing the cells with 1% NP-40 in PBS/0.2% of
BSA. Cell lysates containing biotin-Ad2 were then
added to ELISA wells which had been precoated with

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1µg/well of polyclonal anti-penton base IgG. Biotin-
Ad2 was then detected by addition of streptavidin-
alkaline phosphatase diluted 1:1000 in PBS/0.5% nonfat
dry milk (Blotto) followed by chromogenic substrate.
5   The total amount of Ad2-biotin bound to the cells was
determined at 4°C in the absence of soluble avidin.
Nonspecific binding (less than 10% of total) was
determined by analyzing cells incubated in the absence
of biotin-Ad2.
10   To analyze soluble penton base internalization,
radiolabeled penton base was added to cells at 4°C and
then immediately warmed to 37°C. At varying times,
samples were taken and diluted 10-fold in ice-cold
HBSE (Hepes-buffered saline containing 10mM EDTA),
15   washed twice in this buffer, and then resuspended in a
small volume of HBSE containing 2mg/ml subtilisin
(Sigma, St. Louis, MO) and incubated at 37°C for 15
minutes. Finally, the cells were washed in ice-cold
adhesion buffer, and the remaining protease-resistant
20   Ad2-associated cpm were measured. To determine the
total cell associated penton base, the samples wee
washed three times in HBS containing 1mM $CaCl_2$ and 1mM
$MgCl_2$. Subtilisin-treated cells remained >95% viable.
To analyze soluble penton base internalization,
25   radiolabeled penton base was added to cells at 4°C and
then immediately warmed to 37°C. At varying times,
samples were taken and diluted 10-fold in ice-cold
HBSE (Hepes-buffered saline containing 10mM EDTA),
washed twice in this buffer, and then resuspended in a
30   small volume of HBSE containing 2mg/ml subtilisin
(Sigma) and incubated at 37°C for 15 minutes.
finally, the cells were washed in ice-cold adhesion
buffer, and the remaining protease-resistant Ad2-
associated cpm were measured. To determine the total

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cell associated penton base, the samples were washed
three times in HBS containing 1mM $CaCl_2$ and 1mM $MgCl_2$.
Subtilisin-treated cells remained >95% viable.

5   Cell permeability was assayed by ^3H-choline
release as previously described (Seth, et al., _J._
Biol. Chem. 260: 9598-9602 (1985)), with minor
modifications. Experiments using CS-1 cells were
performed in suspension, while permeability studies
with other cell liens were performed in monolayer

10   cultures. The appropriate number of cells in adhesion
buffer containing 1-5 mCi/ml ^3H-choline (Amersham
Corp.) were incubated for 1 hour at 37°C. Cells (1 X
10^5 cells/sample) were then washed three times with
ice-cold virus binding buffer (5mM) (Hepes buffered

15   saline, pH 7.0, containing 0.2% BSA, 1mM $CaCl_2$, 1mM
$MgCl_2$, and 50mM NaN_3 to prevent virus internalization
during warming) and incubated for 1 hour at 4°C with
the appropriate virus concentration (0-50 μg/ml for
dosage experiments and 0-10 μg/ml for pH and kinetic

20   experiments). After virus binding, the cell samples
were washed once with saline and then incubated for 1
hour at 37°C with 200ml permeability buffer 50mM MES
(Sigma)-buffered saline, pH 6.0, containing 0.2% BSA,
1mM $CaCl_2$, 1mM $MgCl_2$, and 50mM NaN_3.

25   For pH studies, a combination of 25mM MES and
25mM HEPES was used to adjust the permeability buffer
to the desired pH. After incubation in permeability
buffer, the cell samples were incubated with or
without adenovirus and then gently centrifuged. The

30   percent ^3H-choline release was determined by measuring
the counts released into the permeability buffer and
the counts remaining in the cell pellet.

For experiments using adherent cells, 48-well
untreated polystyrene plates were precoated with

10mg/ml of laminin, vitronectin, or penton base in a
volume of 0.2ml. Wells were then blocked with 5% BSA
and then 10^5 HEp-2 or CS-1 cells in adhesion buffer
containing 1-5 mCi/ml [^{3}H]-choline were added to each
5   well. Cells became adherent within 1 hour, ter which
they were washed three times with virus binding buffer
and then incubated with adenovirus for 1 hour at 4°C.
Cells were then washed once with saline and incubated
with permeability buffer for 1 hour at 37°C. ^{3}H-
10 choline release was determined by measuring the
radioactivity in the buffer and in the adherent cells
which were solubilized in 0.2ml of 1% SDS. For
blocking experiments, antibodies or other soluble
proteins were incubated with adherent cells for 1 hour
15 prior to virus binding and in the subsequent virus
binding and low pH incubations.

                    b.   Results

       In initial studies, the potential involvement of
α_v integrins in Ad2-induced membrane permeabilization
20 was analyzed. It was reasoned that if integrins $\alpha_v\beta_3$
and $\alpha_v\beta_5$ were involved in virus-induced membrane
permeabilization, then cells adhered to immobilized
vitronectin or penton base protein should be resistant
to Ad-2 mediated membrane permeabilization since their
25 α_v integrins would be redistributed to the basolateral
surface and thus unavailable to the virus. Cell
permeabilization studies were performed in the
presence of 50mM NaN_3 to prevent virus
internalization, thus restricting virus interactions
30 to the cell plasma membrane.

       Human epithelial Hep-2 cells adhered to
immobilized vitronectin or penton base were
significantly less susceptible to Ad2-induced membrane
permeabilization at pH 6.0 compared to cells adhered

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to a control matrix protein, laminin, which binds to
non-α_v integrins (data not shown). In parallel
experiments (not shown), it was found that
approximately 50% of the total α_v integrins were
5   redistributed to the basolateral surface of cells
adhered to vitronectin or penton base but not on cells
adhered to laminin as indicated by the reduction in
binding of ^{125}I-labeled mAb LM142. These studies
strongly suggested that penton base binding to α_v
10   integrin promotes Ad2-induced cell permeabilization.

To further examine this possibility, inhibition
studies using soluble adenovirus capsid proteins or
cell matrix molecules were performed (not shown).
Preincubation of HEp2 cells with soluble penton base
15   significantly inhibited Ad2-induced membrane
permeabilization, while control proteins including the
Ad2 hexon protein of the cell matrix protein had no
effect on Ad2 membrane permeabilization.

In further studies, it was found that a number of
20   cell liens varied in their susceptibility to Ad2-
induced membrane permeabilization and that differences
in susceptibility correlated with the relative levels
of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ expressed on each cell line (not
shown). Therefore, to systematically investigate the
25   role of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in the early events in
Ad2 infection, we established CS-1 cell lines
expressing either $\alpha_v\beta_3$ or $\alpha_v\beta_5$, as described in Section
1 above. CS-1 cells expressing $\alpha_v\beta_3$ (CS-1:ß3) or $\alpha_v\beta_5$
(CS01:ß5) were established by transfecting CS-1 cells
30   with cDNAs encoding the ß3 or ß5 subunit. Transfected
cells were capable of adhering to immobilized
vitronectin or penton base (not shown). Adhesion to
penton base or vitronectin was blocked by the
appropriate mAb to $\alpha_v\beta_3$ (LM609) or $\alpha_v\beta_5$ (P3G2) but not

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by a control antibody to ß1 integrins (P4C10). The
parental cell line, CS-1, failed to adhere to either
penton base or vitronectin (data not shown). (See
also Thomas, et al., _J. Cell. Sci. 105_: 191-201
(1993).)

The penton base binding and membrane-
permeabilization-promoting properties of $\alpha_v\beta_3$ and $\alpha_v\beta_5$
were further examined as follows. Since M21-L4 cells
express much higher levels of $\alpha_v\beta_3$ than $\alpha_v\beta_5$ on their
cell surface, specific and saturable binding of penton
base to M21-L4 cells was observed at both pH 7.4 and
5.5 (kd = 75 vs. 55 nM; data not shown). Five-fold
more penton base binding sites were detected on M21-L4
cells at pH 7.4 than pH 5.5. Penton base binding to
M21-L4 cells at low pH was specific, since it could be
blocked by soluble penton base and by chelation with
EDTA (not shown). Since M21-L4 cells express a 20-
fold higher level of $\alpha_v\beta_3$ than $\alpha_v\beta_5$, these results
suggested that penton base binding at low pH was
mediated by $\alpha_v\beta_3$. Interestingly, the binding of penton
base to M21-L4 at low pH was not inhibited by RGD
peptides, which suggests that the RGD sequence may
have a limited role in binding at low pH.

Differences in penton base binding to $\alpha_v\beta_3$ and $\alpha_v\beta_5$
were also observed by passing solubilized cell
membrane proteins over a penton base affinity column
(data not shown). Under these conditions, $\alpha_v\beta_3$ was
eluted with soluble RGD-containing peptide (e.g.,
GRGDSP, Telios Pharmaceuticals, Inc., San Diego, CA).
In contrast, $\alpha_v\beta_5$ could only be eluted from the penton
base affinity column with EDTA. These results further
indicated a difference in penton base binding to $\alpha_v\beta_3$
and $\alpha_v\beta_5$. To further substantiate this finding, penton
base binding to CS-1:ß3 and CS-1:ß5 cells at varying

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pH was examined (data not shown). Penton base binding
to CS-1:ß5 cells was relatively unaffected by changes
in pH; approximately 60% of penton base binding was
retained at pH 5.5-6.0. In contrast, penton base
binding to CS-1:ß3 cells was significantly inhibited
by decreasing pH and was completely abolished at pH
5.5. These studies suggest that the structural
requirements for penton base binding to $\alpha_v\beta_5$ differ
from those for binding to $\alpha_v\beta_3$. Of particular
relevance, the low pH environment present in cell
endosomes favors the interaction of penton base with
$\alpha_v\beta_5$.

     C.   Targeting Therapeutic Agents to Specific
            Cells or Receptors

     Based on the foregoing disclosure, it is now
evident that therapeutic agents (e.g., therapeutic
nucleotide sequences) of the present invention may be
designed to specifically target particular cell or
tissue types via the alteration and refinement of
particular parameters. For instance, variation of pH
conditions in the delivery vehicle -- particularly if
said vehicle is a liposome -- may preferentially
direct the vehicle to cells expressing a particular
receptor and may further facilitate entry of the
vehicle and its attached or enclosed therapeutic agent
into the cell. Thus, for example, administration of a
therapeutic agent in conjunction with a delivery
vehicle in a low pH environment may promote the
interaction of the agent and vehicle (e.g., penton
base + therapeutic nucleotide sequence + liposomes)
with cells expressing vitronectin receptors,
particularly $\alpha_v\beta_5$ receptors.

     As discussed above, vitronectin receptor ligands
such as the penton base are expected to be

particularly useful in delivering therapeutic agents
to, and into, integrin-expressing cells --
particularly, cells expressing vitronectin receptors.
Further, in view of the finding that different
5   integrins, including vitronectin receptors, are
expressed on different cell types and even in
different locations on cells, this information is
useful in designing targeting and delivery agents
possessed of an elegant selectivity. (See, e.g.,
10   Hynes, Cell 69: 11-25 (1992).) For example, since
adenovirus-derived proteins such as penton base or
complex include one or more RGD sequences,
compositions including penton base and a therapeutic
agent may be preferentially targeted to integrin
15   receptor-expressing cells, such as matrix proteins
involved in inflammation, wound healing, and
development. More particularly, such a composition is
contemplated to be useful in targeting cells
expressing $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrins, or both.

20   Not only would such compositions be useful in the
treatment of inflammatory disorders such as lupus
erythematosus, rheumatoid arthritis, and the like,
such compositions would be useful in targeting
specific malignant, tumorigenic and transformed cells.
25   For example, as integrins have been implicated in
tumor progression and metastasis (Damjanovich, et al.,
Am J. Respir. Cell Mol. Biol. 6: 197-206 (1992);
Ruoslahti, et al., Cancer Cells 1: 119-126 (1989)),
differential expression of integrins on different cell
30   lines will facilitate specific targeting. One example
is provided by lung carcinoma; cell lines of the
phenotype SK-LU-1 express vitronectin receptors (e.g.
$\alpha_v\beta_3$) (Mette, et al., Am J. Respir. Cell Mol. Biol. 8:
562-572 (1993)) and would thus be receptive to

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targeting and delivery by compositions including a
vitronectin receptor ligand such as adenovirus penton
base.

5   Targeting agents directed to other integrins --
e.g. laminin, collagen, fibronectin -- are thus
contemplated to be useful as disclosed herein, as
well. (See, e.g., Albelda, et al., _Lab. Invest._ _68_:
4-17 (1993); Leavesley, et al., _J. Cell Biol._ _117_:
10   1101-1107 (1992); Cheresh, et al., _Cell 57_: 59-69
(1989); and Felding-Haberman, et al., _J. Clin. Invest._
_89_: 2018-2022 (1992).)

The foregoing specification, including the
specific embodiments and examples, is intended to be
15   illustrative of the present invention and is not to be
taken as limiting. Numerous other variations and
modifications can be effected without departing from
the true spirit and scope of the present invention.

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SEQUENCE LISTING

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    (ii) TITLE OF INVENTION: TARGETING AND DELIVERY OF GENES AND
          ANTIVIRAL AGENTS INTO CELLS BY THE ADENOVIRUS PENTON

    (iii) NUMBER OF SEQUENCES: 7

    (iv) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

    (v) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER: PCT/US 94/
        (B) FILING DATE: 03-FEB-1994

    (vi) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 08/015,225
        (B) FILING DATE: 09-FEB-1993

    (vi) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 08/046,159
        (B) FILING DATE: 13-APR-1993

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: internal

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    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

    His Ala Ile Arg Gly Asp Thr Phe Ala
    1   5

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 23 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTTCTAGAAG TATGCAGCGC GCG   23

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 23 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTCTAGATC AAAAAGTGCG GCT   23

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

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    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAAGGATCCA GCTGATGAAA CGCGCCA   27

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTTGGTACCA GCTGTTATTC CTGGGCA   27

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: internal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

    Glu Asp Pro Gly Phe Phe Asn Val Glu
    1   5

(2) INFORMATION FOR SEQ ID NO:7:

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(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 11 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Glu Asp Pro Gly Lys Gln Leu Tyr Asn Val Glu
1   5   10

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Claims:

    1.   A composition designed to specifically target epithelial cells and deliver a therapeutic nucleotide sequence to said cells, comprising an adenovirus-derived protein and said nucleotide sequence.

    2.   The composition of claim 1, wherein said protein is selected from the group consisting of penton base and penton complex.

    3.   The composition of claim 1, wherein said protein is conventionally-purified.

    4.   The composition of claim 1, wherein said protein is recombinant.

    5.   The composition of claim 1, wherein said protein is derived from adenovirus type 2.

    6.   The composition of claim 1, wherein said therapeutic nucleotide sequence is operatively linked to said adenovirus-derived protein.

    7.   The composition of claim 1, wherein said therapeutic nucleotide sequence and said adenovirus-derived protein are contained within a liposome.

    8.   The composition of claim 1, wherein said nucleotide sequence encodes a polypeptide and further comprises an active promoter for expressing said polypeptide.

    9.   The composition of claim 1, wherein said nucleotide sequence is an "antisense" sequence.

    10.   The composition of claim 1, further comprising a pharmaceutically acceptable carrier or excipient.

    11.   Use of a composition comprising an adenovirus-derived protein and a therapeutic nucleotide sequence in the manufacture of a medicament for specifically targeting epithelial cells and delivering an effective amount of said therapeutic

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nucleotide sequence to said cells.

12. The use of claim 11, wherein said
therapeutic nucleotide sequence and said adenovirus-
derived protein are operatively linked.

13. The use of claim 11, wherein said protein is
selected from the group consisting of penton base and
penton complex.

14. The use of claim 11, wherein said protein is
conventionally purified.

15. The use of claim 11, wherein said protein is
recombinant.

16. The use of claim 11, wherein said protein is
derived from adenovirus type 2.

17. The use of claim 11, wherein said nucleotide
sequence and said protein are incorporated into a
liposome.

18. A method of making a medicament useful in
specifically targeting and delivering a therapeutic
nucleotide sequence to mammalian cells, comprising
operatively linking an adenovirus-derived amino acid
residue sequence and said nucleotide sequence and
incorporating said operatively linked sequences into a
liposome.

19. The method of claim 18, wherein said amino
acid residue sequence comprises penton base or penton
complex.

20. An article of manufacture comprising
packaging material and a composition effective for
targeting epithelial cells and delivering a
therapeutic nucleotide sequence to said cells, wherein
said composition comprises an adenovirus-derived
protein and a therapeutic nucleotide sequence, and
wherein said packaging material comprises a label
which indicates that the composition can be used for
targeting epithelial cells and delivering a

therapeutic nucleotide sequence to said cells.

II — HEXON
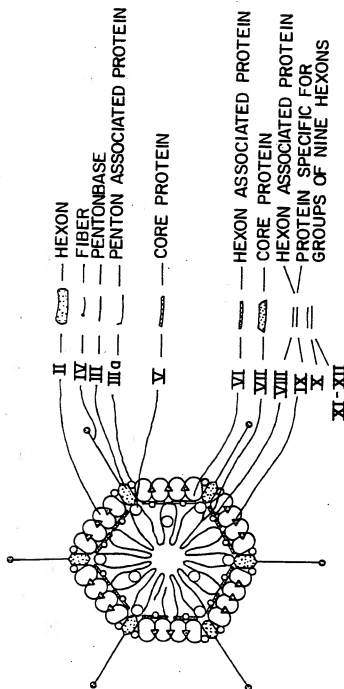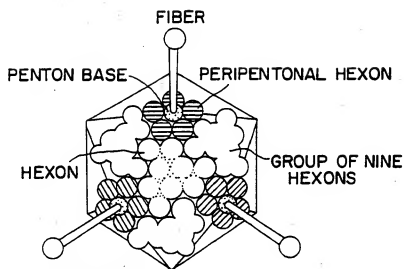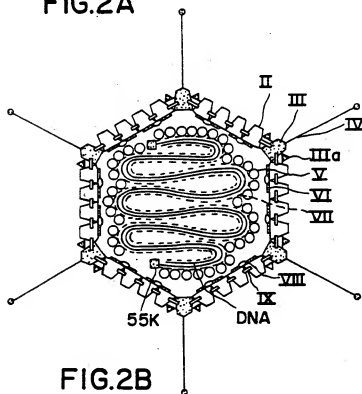IV — FIBER
III — PENTONBASE
IIIa — PENTON ASSOCIATED PROTEIN

V — CORE PROTEIN

VI — HEXON ASSOCIATED PROTEIN
VII — CORE PROTEIN
VIII — HEXON ASSOCIATED PROTEIN
IX — PROTEIN SPECIFIC FOR
X — GROUPS OF NINE HEXONS
XI - XII

FIG.I

FIBER

PENTON BASE   PERIPENTONAL HEXON

HEXON   GROUP OF NINE
                                      HEXONS

FIG.2A

II

III

IV

IIIa

V

VI

VII

VIII

IX

55K   DNA

FIG.2B
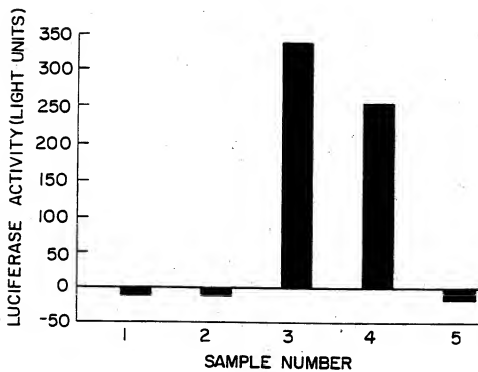
FIG.3

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 48/00; C12N 5/22, 7/04, 15/34, 15/88

US CL : 514/44, 435/69.1, 236; 935/54, 62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44, 435/69.1, 236; 935/54, 62; 930/220

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

FUNDAMENTAL VIROLOGY

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,166,320 (Wu et al) 24 November 1992, see entire document.	1-20
Y	Journal of Virology, Volume 64, No. 8, issued August 1990, Defer et al, "Human Adenovirus-Host Cell Interactions: Comparative Study with Members of Subgroups B and C", pages 3661-3673, see entire document.	1-20
Y	PNAS USA, Vol. 88, issued October 1991, Curiel et al, "Adenovirus enhancement of transferrin-polylysine-mediated gene delivery", pages 8850-8854, see entire document.	1-20
Y	Journal of Virology, Volume 56, Number 2, issued November 1985 Jan van Oostrum et al. "Molecular Composition of the Adenovirus Type 2 Virion"	439-448 pages the entire document.

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	
"E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 APRIL 1994	04 MAY 1994

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